IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.53(b)

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Dear Sir:

This is a request for filing a **DIVISIONAL** application under 37 CFR 1.53(b) of pending prior application Serial No. 09/196,480, filed on November 19, 1998, which is a divisional application of U.S. application Serial No. 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393, both entitled A NOVEL HUMAN JAK2 KINASE.

1. __X__ Enclosed is a copy of the prior application, U.S. Application Serial No. 08/567,508 filed December 5, 1995, including the oath or declaration as originally signed.

2. __X__ With regard to the requirement of 37 CFR 1.821(e) which requires that a copy of the Sequence Listing in computer readable form (CRF) be submitted, Applicants state that the computer readable form of the "Sequence Listing" for the instant divisional application is identical with the substitute sequence listing filed December 11, 1997, for Serial No. 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393, to which priority is claimed. In accordance with 37 C.F.R. §1.821(e), please use the computer readable form filed with the December 11, 1997 substitute sequence listing, as the computer readable form for the instant divisional application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant divisional application.

3. __X__ Cancel in this application original claims 2, 3, and 11-13 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

4. __X__ The inventors of the invention being claimed in this application are: Roger Coleman and Susan G. Stuart.

5. _X_ In accordance with 37 CFR 1.63(d), a copy of the originally signed declaration showing applicants' signatures as filed on March 20, 1996 is enclosed.

6. _X_ Amend the specification by inserting before the first line the sentence: "This application is a divisional application of U.S. application serial number 09/196,480, filed November 19, 1998, which is a divisional of U.S. application serial number 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393."

7. _X_ The filing fee is calculated below:

Claims	Number Filed	Minus	Number Extra	Other Than Small Entity Rate	Fee	Basic Fee $760.00
Total Claims	13	-20	0	x $18		$ 0
Indep. Claims	1	-3	0	x $78		$ 0
__ Multiple Dependent Claim(s), if any				+ $260		$ 0

TOTAL FILING FEE $ 760.00

8. ___ An extension of time in the above-named prior application has been requested and the fees therefore have been authorized in said application.

9. _X_ Please charge Incyte Pharmaceuticals, Inc. Deposit Account No. **09-0108** in the amount of $760.00 .

The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to Incyte Pharmaceuticals, Inc. Account No. **09-0108**.

A **duplicate** copy of this Request is enclosed.

10. _X_ New formal drawings are enclosed.

11. _X_ The prior application is assigned of record to Incyte Pharmaceuticals, Inc, recorded on March 25, 1996, at reel 7984/frame 0461.

12. _X_ A preliminary amendment is enclosed.

13. _X_ Also enclosed Return Postcard, Information Disclosure Statement, List of Cited References (1449), copy of previously submitted Certificate, Revocation of Power of Attorney and Appointment of New Attorneys, Submission of Formal Drawings, copy of Substitute Submission Under 37 C.F.R. 1.821-1.825, and copy of previously submitted Substitute Sequence Listing.

2

14. __X__   The power of attorney of the prior application is to:

Narinder S. Banait	**Reg. No. 43,482**
Adam Warwick Bell	**Reg. No. 43,490**
Lucy J. Billings	**Reg. No. 36,749**
Michael C. Cerrone	**Reg. No. 39,132**
Diana Hamlet-Cox	**Reg. No. 33,302**
Colette C. Muenzen	**Reg. No. 39,784**
Lynn E. Murry	**Reg. No. 42,918**
Danielle M. Pasqualone	**Reg. No. 43,847**
Susan K. Sather	**Reg. No. 44,316**
David G. Streeter	**Reg. No. 43,168**

a. ___   An associate power of attorney is attached.

b. ___   Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. __X__   Address all future correspondence to:

INCYTE PHARMACEUTICALS, INC.
PATENT DEPARTMENT
3174 Porter Drive
Palo Alto, California 94304
Phone: (650) 855-0555, Fax: (650) 849-8886

Date: _December 9, 1999_   By: _Susan K. Sather_

Susan K. Sather
Reg. No. 44,316
Direct Dial Telephone: (650) 845-4646

___ Inventor(s)
___ Assignee of complete interest
__X__ Attorney or agent of record
___ Filed under 37 CFR 1.34(a)
Registration number if acting under 37 CFR 1.34(a) _____.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
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1

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Susan K. Sather	Reg. No. 44,316
David G. Streeter	Reg. No. 43,168

a. ___ An associate power of attorney is attached.

b. ___ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. __X__ Address all future correspondence to:

<div align="center">

INCYTE PHARMACEUTICALS, INC.
PATENT DEPARTMENT
3174 Porter Drive
Palo Alto, California 94304
Phone: (650) 855-0555, Fax: (650) 849-8886

</div>

Date: _December 9, 1999_   By: _Susan K. Sather_

Susan K. Sather
Reg. No. 44,316
Direct Dial Telephone: (650) 845-4646

___ Inventor(s)
___ Assignee of complete interest
__X__ Attorney or agent of record
___ Filed under 37 CFR 1.34(a)
  Registration number if acting under 37 CFR 1.34(a) _____.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: A NOVEL HUMAN JAK2 KINASE

Serial No.:	To Be Assigned	Filing Date:	Herewith
Examiner:	To Be Assigned	Group Art Unit:	To Be Assigned

Assistant Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified application as indicated below.

IN THE SPECIFICATION

Please amend the specification as follows:

At page 1, line 7, replace "prevention" with --prevention,--,

At page 1, line 15, replace "Jak3" with --Jak3,--.

At page 1, line 16, replace "addition" with --addition,--.

At page 3, line 23, replace "acid acid" with --acid--.

At page 3, line 24, replace "prevention" with --prevention,--.

At page 3, line 30, replace "(SEQ ID No 1)" with --(SEQ ID NO: 1)--.

At page 3, line 30 replace "(SEQ ID No 2)" with --(SEQ ID NO: 2)--

At page 3, line 30, replace "hjak2," with --hjak2--.

At page 3, line 30, delete "(SEQ ID No 2),".

At page 3, line 31, replace "HJAK2" with -- HJAK2 (SEQ ID NO:2)--.

At page 3, line 33, replace "86:1603-7)" with --86:1603-7),--.

At page 4, line 1, replace "hjak2, SEQ ID No1" with --hjak2 (SEQ ID NO:1)--.

At page 5, line 2, replace "Figure 1 displays" with --Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, and 1H display--.

At page 5, line 2, replace "nucleic acid and amino acid" with --nucleic acid (SEQ ID NO:1) and amino acid (SEQ ID NO:2)--.

At page 5, line 3, replace "this and the" with --these and in the--.

At page 5, line 4, replace "figure" with --figures--.

At page 5, line 5, replace "Inc" with --Inc.--.

At page 5, line 6, replace "Figure 2 shows" with --Figures 2A, 2B, 2C, 2D, and 2E show--.

At page 5, lines 6 through 7, replace "MUSPTK1 GI and HJAK2" with --HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3)--.

At page 5, line 12, replace "RNA" with --RNA,--.

At page 5, line 12, replace "sequence" with --sequence,--.

At page 8, lines 10 and 11, replace "a "fragment ", "portion ", or "segment" of" with --a "fragment", a "portion", or a "segment" of--.

At page 8, line 25, replace "etc" with --etc.--.

At page 8, line 26, replace "etc) or" with --etc.),--.

At page 8, line 26, replace "simians, etc)" with --simians, etc.)--.

At page 9, line 22, replace "an" with --a--.

At page 9, line 28, replace "86:1603-7)" with --86:1603-7),--.

At page 10, line 2, replace "including but not limited to" with --including, but not limited to,--.

At page 10, line 5, replace "produced and some" with --produced. Some--.

At page 10, line 20, delete "(REF)".

At page 10, line 28, replace "NY" with --NY, Chapters 4, 8, 16, and 17--.

At page 10, line 30, replace "City) ." with --New York NY, Chapters 9, 13, and 16).--.

At page 11, line 1, replace "PCR" with --PCR,--.

At page 11, line 6, replace "antisense" with --antisense orientation--.

At page 11, line 13, replace "Gobinda et al" with --Sarkar--.

At page 11, line 20, replace "Gobinda et al" with --Sarkar--.

At page 11, line 35, replace "Gobinda et al" with --Sarkar--.

At page 12, line 8, replace "PromoterFinder™" with --PROMOTERFINDER--.

At page 12, line 16, replace "XL-PCR™" with --XL-PCR--.

At page 12, line 26, replace "In step 2," with --In the second step,--.

At page 12, lines 27 and 28, replace "Steps 3 and 4," with --The third and fourth steps,--.

At page 12, line 29, replace "Step 5," with --The fifth step,--.

At page 12, line 30, replace "In step 6," with --The sixth step,--.

At page 13, line 16, replace "devise" with --device--.

At page 13, line 18, replace "Genotyper™" with --GENOTYPER--.

At page 13, line 18, replace "Sequence Navigator™" with --SEQUENCE NAVIGATOR--.

At page 13, line 36, replace "NO 1" with --NO:1--.

At page 14, line 9, replace "T7, T3 or SP6 and labelled" with --T7, T3, or SP6, and labeled--.

At page 16, line 29, after "using" insert --an--.

At page 16, line 30, replace "Peptide Synthesizer" with --peptide synthesizer--.

At page 17, line 32, replace "Library" with --The cDNA library--.

At page 17, lines 32 and 33, delete "obtained from Mayo Clinic".

At page 18, line 5, replace "Uni ZAP™" with --UNIZAP--.

At page 18, line 7, replace "pBluescript™" with --PBLUESCRIPT--.

At page 18, line 7, replace "library" with --library,--.

At page 18, line 8, replace ", and" with --and--.

At page 18, line 10, replace "XL1-Blue™" with --XL1-BLUE--.

At page 18, line 10, replace "pBluescript" with --PBLUESCRIPT--.

At page 18, line 20, replace "Plasmid Purification System" with --plasmid purification system--.

At page 18, line 25, replace "Catalyst" with --CATALYST--.

At page 18, line 26, replace "Hamilton Micro Lab" with --MICROLAB--.

At page 18, line 27, replace "Thermal Cyclers" with --thermal cyclers--.

At page 18, line 28, replace "Sequencing Systems" with --sequencing systems--.

At page 18, line 35, replace "Sequenase®" with --SEQUENASE--.

At page 18, line 35, replace "Corp)" with --Corp.,--.

At page 19, line 5, delete "Hamilton".

At page 19, line 6, replace "Micro Lab" with --MICROLAB--.

At page 19, line 6, replace "Thermal Cycler" with --thermal cycler--.

At page 19, line 7, replace "Catalyst" with --CATALYST--.

At page 19, line 19, replace "INHERIT™ 670 Sequence Analysis System" with --INHERIT 670 sequence analysis system--.

At page 19, line 31, replace "INHERIT™" with --INHERIT--.

At page 20, line 24, replace "Fig 1" with --Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, and 1H--.

At page 20, line 24, replace "Fig 2" with --Figures 2A, 2B, 2C, 2D, and 2E--.

At page 20, line 37, replace "Oligo" with --OLIGO--.

At page 21, lines 5 and 6, replace "XLR = GGGCGGAAGTGCTCTCGGCGGAAG" with --XLR (SEQ ID NO:4)--.

At page 21, line 6, replace "XLF = AGTGTGCTACAGTGCTGGTCGTCG" with --XLF (SEQ ID NO:5)--.

At page 21, line 13, replace "Thermal Cycler" with --thermal cycler--.

At page 21, line 35, replace " QIAQuick™" with --QIAQUICK--.

At page 22, line 34, replace "229-36" with --212:229-236--.

At page 23, line 17, replace "Oligo 4.0" with --OLIGO 4.0 software--.

At page 23, line 20, replace "Inherit Analysis" with --INHERIT analysis--.

At page 24, line 4, replace "a organ, tumor, synovial cavity" with --an organ, a tumor, a synovial cavity,--.

At page 24, line 8, replace "and three" with --and for three--.

At page 24, line 12, replace "tissue" with --tissue,--.

At page 24, line 19, replace "tissue" with --tissue,--.

At page 25, line 9, replace "then used" with --then be used--.

At page 25, line 33, replace "tac" with --tac)--.

At page 25, line 34, replace "oxidase" with --oxidase,--.

At page 27, line 35, replace "regions, as shown in Fig. 3," with --regions--.

At page 28, line 1, replace "N-terminus" with --N-terminus,--.

At page 28, line 2, delete "those".

At page 28, line 5, replace "peptides," with --peptides of--.

At page 28, line 5, replace "length," with --length--.

At page 28, line 6, replace "Peptide Synthesizer Model 431A" with "431A peptide synthesizer--.

At page 30, line 8, replace "Sepharose" with --SEPHAROSE--.

Please replace pages 35 through 43 with substitute pages 35 through 43, which accompany this Preliminary Amendment.

IN THE DRAWINGS

Please amend Figures 2A, 2B, 2C, 2D, 2E, and 2F as shown in red on the attached sheets.

Please replace Figures 1A, 1B, 1C, 1D, 1E, and 1F and Figures 2A, 2B, 2C, 2D, 2E, 2F as filed with the formal drawings which accompany this Preliminary Amendment.

IN THE CLAIMS

Please cancel claims 2, 3 and 11-13.

REMARKS

Justification for the amendments is as follows. Claims 2, 3, and 11-13 are canceled above, and claims 1, 4-10, and 14-18 are pending.

The amendment to the specification at pages 35 through 43 of the specification replaces the Sequence Listing as filed with the Substitute Sequence Listing filed December 11, 1997, in prior U.S. application Serial No. 08/567,508, and referenced in the filing papers for the present application. (See page 1 of the transmittal for the present application.) The Substitute Sequence Listing properly identifies the MUSPTK1 sequence, described at page 5 of the specification and shown in Figures 2A, 2B, 2C, 2D, 2E, and 2F as originally filed, as SEQ ID NO:3, and identifies sequences XLR and XLF, which appear at page 21 of the specification as originally filed, as SEQ ID NO:4 and SEQ ID NO:5, respectively. These sequences do not constitute new matter as MUSPTK1, XLR, and XLF appear in the application as filed.

The Substitute Sequence Listing corrects an error in SEQ ID NO:2 in the Sequence Listing as originally filed. Specifically, the latter part of SEQ ID NO:2, starting around residue 990, appears to have been incorrectly translated. However, SEQ ID NO:2 was correct as shown in Figures 1A, 1B, 1C, 1D, 1E, and 1F as filed. Accordingly, SEQ ID NO:2 as it appears in the Substitute Sequence Listing accompanying this Amendment is correct. This corrected sequence is not new matter as the correct sequence was shown in Figures 1A, 1B, 1C, 1D, 1E, and 1F of the application as filed. Applicants apologize for any inconvenience that might have been caused by this error. Applicants note that SEQ ID NO:1 was correct in the application as filed in both Figures 1A, 1B, 1C, 1D, 1E, and 1F and in the Sequence Listing.

The amendments to the specification at page 5, lines 2 and 6, and page 20, line 24, are made to reflect the renumbering of the figures in the preparation of formal drawings. The remaining amendments to the specification are merely typographical or grammatical in nature.

The amendments to the drawings were made to clarify Figures 2A, 2B, 2C, 2D, and 2E, and 2F. Specifically, Figures 2A, 2B, 2C, 2D, 2E, and 2F as originally filed showed the alignment between HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3), along with two consensus sequences generated by DNASTAR software, the multisequence alignment program used. Figures 2A, 2B, 2C, 2D, 2E, and 2F as amended show only the alignment

between HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3).

No new matter is added by any of these amendments.

If there are any questions regarding the above, the Examiner is invited to call Applicants' Agent at (650) 855-0555.

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Date: *December 9, 1999*　　　　　*Susan K. Sather*

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        M G M A C L T M T E M E . T S T S . . . Q N G D I . G . A N
        M G M A C L T M T E M E G T S T S S V H Q N G D I S G S A N

                    10                  20                  30

   1    M G M A C L T M T E M E A T S T S P V H Q N G D I P G S A N
   1    M G M A C L T M T E M E G T S T S S I Y Q N G D I S G N A N


        S . K Q I . P V L Q V Y L Y H S L G . . E . . Y L . P P S G
        S V K Q I D P V L Q V Y L Y H S L G Q A E G D Y L T P P S G

                    40                  50                  60

  31    S V K Q I E P V L Q V Y L Y H S L G Q A E G E Y L K P P S G
  31    S M K Q I D P V L Q V Y L Y H S L G K S E A D Y L T P P S G


        E Y V . E E I C . A A S K A C G I T P V Y H N M F A L M S E
        E Y V G E E I C V A A S K A C G I T P V Y H N M F A L M S E

                    70                  80                  90

  61    E Y V A E E I C V A A S K A C G I T P V Y H N M F A L M S E
  61    E Y V G E E I C I A A S K A C G I T P V Y H N M F A L M S E


        T E R I W Y P P N H V F H I D E S T R H . . L Y R I R F Y F
        T E R I W Y P P N H V F H I D E S T R H D V L Y R I R F Y F

                    100                 110                 120

  91    T E R I W Y P P N H V F H I D E S T R H D I L Y R I R F Y F
  91    T E R I W Y P P N H V F H I D E S T R H N V L Y R I R F Y F


        P . W Y C S G S . R . Y R . G . . S R G A E A P L L D D F V M
        P H W Y C S G S S R A Y R H G V S R G A E A P L L D D F V M

                    130                 140                 150

 121    P H W Y C S G S S R T Y R Y G V S R G A E A P L L D D F V M
 121    P R W Y C S G S N R A Y R H G I S R G A E A P L L D D F V M


        S Y L F . Q W R H D F V H G W I K V P V T H E T Q E E C L G
        S Y L F A Q W R H D F V H G W I K V P V T H E T Q E E C L G

                    160                 170                 180

 151    S Y L F V Q W R H D F V H G W I K V P V T H E T Q E E C L G
 151    S Y L F A Q W R H D F V H G W I K V P V T H E T Q E E C L G


        M . V L D M M R I A K E . D Q T P L A . Y N S . S Y K T F L
        M A V L D M M R I A K E N D Q T P L A V Y N S V S Y K T F L

                    190                 200                 210

 181    M A V L D M M R I A K E K D Q T P L A V Y N S V S Y K T F L
 181    M T V L D M M R I A K E N D Q T P L A I Y N S I S Y K T F L
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FIGURE 2A

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      P . C . R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
      P Q C V R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
                        220                 230                 240
211   P K C V R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
211   P Q C I R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F

      S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E . F
      S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F
                        250                 260                 270
241   S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F
241   S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E K F

      E V K E . . . . G P S G E E I F A T I I I T G N G G I Q W S R
      E V K E S G S G P S G E E I F A T I I I T G N G G I Q W S R
                        280                 290                 300
271   E V K E S A R G P S G E E I F A T I I I T G N G G I Q W S R
271   E V K E P G S G P S G E E I F A T I I I T G N G G I Q W S R

      G K H K E S E T L T E Q D . Q L Y C D F P . I I D V S I K Q
      G K H K E S E T L T E Q D V Q L Y C D F P D I I D V S I K Q
                        310                 320                 330
301   G K H K E S E T L T E Q D V Q L Y C D F P D I I D V S I K Q
301   G K H K E S E T L T E Q D L Q L Y C D F P N I I D V S I K Q

      A N Q E . S N E S R . V T . H K Q D G K . L E I E L S S L .
      A N Q E G S N E S R V V T V H K Q D G K V L E I E L S S L K
                        340                 350                 360
331   A N Q E C S N E S R I V T V H K Q D G K V L E I E L S S L K
331   A N Q E G S N E S R V V T I H K Q D G K N L E I E L S S L R

      E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
      E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
                        370                 380                 390
361   E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
361   E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
```

FIGURE 2B

```
        A V L E N I . S N C H G P I S M D F A I S K L K K A G N Q T
        A V L E N I Q S N C H G P I S M D F A I S K L K K A G N Q T
                     400           410           420

391     A V L E N I H S N C H G P I S M D F A I S K L K K A G N Q T
391     A V L E N I Q S N C H G P I S M D F A I S K L K K A G N Q T


        G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
        G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
                     430           440           450

421     G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
421     G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K


        H C L I T K N E N . E Y N L S G T . . N F S . L K D L L N C
        H C L I T K N E N G E Y N L S G T N K N F S S L K D L L N C
                     460           470           480

451     H C L I T K N E N G E Y N L S G T N R N F S N L K D L L N C
451     H C L I T K N E N E E Y N L S G T K K N F S S L K D L L N C


        Y Q M E T V R S D . I I F Q F T K C C P P K P K D K S N L L
        Y Q M E T V R S D S I I F Q F T K C C P P K P K D K S N L L
                     490           500           510

481     Y Q M E T V R S D S I I F Q F T K C C P P K P K D K S N L L
481     Y Q M E T V R S D N I I F Q F T K C C P P K P K D K S N L L


        V F R T N G . S D V . . S P T L Q R . . . . N Q M V F H K I
        V F R T N G V S D V Q I S P T L Q R H T N V N Q M V F H K I
                     520           530           540

511     V F R T N G I S D V Q I S P T L Q R H N N V N Q M V F H K I
511     V F R T N G V S D V P T S P T L Q R P T H M N Q M V F H K I


        R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
        R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
                     550           560           570

541     R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
541     R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y


        G Q L H . T E V L L K V L D K A H R N Y S E S F F E A A S M
        G Q L H E T E V L L K V L D K A H R N Y S E S F F E A A S M
                     580           590           600

571     G Q L H K T E V L L K V L D K A H R N Y S E S F F E A A S M
571     G Q L H E T E V L L K V L D K A H R N Y S E S F F E A A S M
```

FIGURE 2C

```
                   610              620              630
601   M S Q L S H K H L V L N Y G V C V C G E E N I L V Q E F V K
601   M S K L S H K H L V L N Y G V C V C G D E N I L V Q E F V K
```

```
                   640              650              660
631   F G S L D T Y L K K N K N S I N I L W K L G V A K Q L A W A
631   F G S L D T Y L K K N K N C I N I L W K L E V A K Q L A W A
```

```
                   670              680              690
661   M H F L E E K S L I H G N V C A K N I L L I R E E D R R T G
661   M H F L E E N T L I H G N V C A K N I L L I R E E D R K T G
```

```
                   700              710              720
691   N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P
691   N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P
```

```
                   730              740              750
721   P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G
721   P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G
```

```
                   760              770              780
751   D K P L S A L D S Q R K L Q F Y E D K H Q L P A P K W T E L
751   D K P L S A L D S Q R K L Q F Y E D R H Q L P A P K W A E L
```

```
                   790              800              810
781   A N L I N N C M D Y E P D F R P A F R A V I R D L N S L F T
781   A N L I N N C M D Y E P D F R P S F R A I I R D L N S L F T
```

FIGURE 2D

```
                      PDYELLTENDMLPNMRIGALGFSGAFEDRD
                      PDYELLTENDMLPNMRIGALGFSGAFEDRD
                          820          830          840
    811  PDYELLTENDMLPNMRIGALGFSGAFEDRD
    811  PDYELLTENDMLPNMRIGALGFSGAFEDRD

                      PTQFEERHLKFLQQLGKGNFGSVEMCRYDP
                      PTQFEERHLKFLQQLGKGNFGSVEMCRYDP
                          850          860          870
    841  PTQFEERHLKFLQQLGKGNFGSVEMCRYDP
    841  PTQFEERHLKFLQQLGKGNFGSVEMCRYDP

                      LQDNTGEVVAVKKLQHSTEEHLRDFEREIE
                      LQDNTGEVVAVKKLQHSTEEHLRDFEREIE
                          880          890          900
    871  LQDNTGEVVAVKKLQHSTEEHLRDFEREIE
    871  LQDNTGEVVAVKKLQHSTEEHLRDFEREIE

                      ILKSLQHDNIVKYKGVCYSAGRRNL.LIME
                      ILKSLQHDNIVKYKGVCYSAGRRNLKLIME
                          910          920          930
    901  ILKSLQHDNIVKYKGVCYSAGRRNLRLIME
    901  ILKSLQHDNIVKYKGVCYSAGRRNLKLIME

                      YLPYGSLRDYLQKHKERIDH.KLLQYTSQI
                      YLPYGSLRDYLQKHKERIDHIKLLQYTSQI
                          940          950          960
    931  YLPYGSLRDYLQKHKERIDHKKLLQYTSQI
    931  YLPYGSLRDYLQKHKERIDHIRLLQYTSQI

                      CKGMEYLGTKRYIHRDLATRNILVENENRV
                      CKGMEYLGTKRYIHRDLATRNILVENENRV
                          970          980          990
    961  CKGMEYLGTKRYIHRDLATRNILVENENRV
    961  CKGMEYLGTKRYIHRDLATRNILVENENRV

                      KIGDFGLTKVLPQDKEYYKVKEPGESPIFW
                      KIGDFGLTKVLPQDKEYYKVKEPGESPIFW
                         1000         1010         1020
    991  KIGDFGLTKVLPQDKEYYKVKEPGESPIFW
    991  KIGDFGLTKVLPQDKEYYKVKEPGESPIFW
```

FIGURE 2E

```
        Y A P . S L T E S K F S V A S D V W S F G V V L Y E L F T Y
        Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y
                    1030              1040              1050
1021    Y A P Q S L T E S K F S V A S D V W S F G V V L Y E L F T Y
1021    Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y

        I E K S K S P P . E F M R M I G N D K Q G Q M I V F H L I E
        I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E
                    1060              1070              1080
1051    I E K S K S P P V E F M R M I G N D K Q G Q M I V F H L I E
1051    I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E

        L L K . N G R L P R P . G C P D E I Y . I M T E C W N N N V
        L L K S N G R L P R P D G C P D E I Y V I M T E C W N N N V
                    1090              1100              1110
1081    L L K S N G R L P R P E G C P D E I Y V I M T E C W N N N V
1081    L L K N N G R L P R P D G C P D E I Y M I M T E C W N N N V

        . Q R P S F R D L . . . . . . I . . . . .
        S Q R P S F R D L A L R V G Q I K D G T A G
                    1120              1130
1111    S Q R P S F R D L S F - - G W I K C G T V .
1111    N Q R P S F R D L A L R V D Q I R D N M A G
```

FIGURE 2F

PF-0049P

1

<div align="center">

A NOVEL HUMAN JAK2 KINASE

FIELD OF THE INVENTION
</div>

The present invention relates to a novel, human Jak2 kinase isolated

6   from human placenta and to the use of this novel protein and its nucleic

acid sequence in the diagnosis, study, prevention and treatment of disease.

<div align="center">

BACKGROUND OF THE INVENTION
</div>

JAK kinases are Janus family nonreceptor protein-tyrosine kinases

11   (NR-PTK) that lack transmembrane regions and form functional complexes with

the intercellular regions of other cell surface receptors. They were first

identified as the products of mutant oncogenes in cancer cells where their

activation was no longer subject to normal cellular controls. Described

JAK kinases include Jak1, Jak2, and Jak3 which all share the conserved

16   kinase domain. In addition these proteins have 5 to 100 amino acid

residues located on either side of, or inserted into loops of, the

carboxyterminal kinase domain which allow the regulation of each kinase as

it recognizes and interacts with its target protein. Known target proteins

include growth hormone receptor, prolactin receptor, erythropoietin

21   receptor, cytokine receptors and others which utilize the common chain

known as gp130. These receptors are unique both in their ability to

recruit multiple PTKs and in the diversity of their responses within

different cell types (Taniguchi T (1995) Science 268:251-55). Genetic

evidence places these kinases in the interferon α and γ signal transduction

26   pathways which are widely expressed in mammalian cells.

Kinases regulate many different cell proliferation, differentiation,

and signaling processes by adding phosphate groups to proteins. The high

energy phosphate which drives activation is generally transferred from

adenosine triphosphate molecules (ATP) to a particular protein by the PTKs,

31   and the transfer process is roughly analogous to turning on a molecular

switch. When the switch goes on, the kinase activates a metabolic enzyme,

regulatory protein, receptor, cytoskeletal protein, ion channel or pump,

transcription factor, or another kinase. For example, in their normal

role, the JAK NR-PTKs are capable of regulating tyrosine phosphorylation of

36   STAT proteins, signal transducers and activators of transcription, such

that they translocate to the nucleus and bind DNA (David M et al. (1995)

<div align="center">1</div>

1   Science 269:1721-1723). In contrast, uncontrolled kinase signaling has been implicated in inflammation, oncogenesis, arteriosclerosis, and psoriasis.

    Almost all kinases contain a similar 250-300 amino acid catalytic domain. The N-terminal domain generally folds into a two-lobed structure

6   to bind and orient ATP (or GTP) donor molecules. The larger C terminal lobe binds the protein substrate and carries out the transfer of phosphate from ATP to the hydroxyl group of a tyrosine residue. The primary structure of the kinase domain is conserved in the residues: G_{50} and G_{52} in subdomain I, K_{72} in subdomain II, G_{91} in subdomain III, E_{208} in subdomain

11   VIII, D_{220} and G_{225} in subdomain IX, and the amino acid motifs of subdomains VIB, VIII and IX (Hardie G and Hanks S (1995) Academic Press, San Diego CA).

    The novel human Jak2 kinase, hjak2, of the present application shows significant conservation of the diagnostic kinase residues which allowed

16   its identification from among the isolated cDNAs of a placenta library, the anatomy and physiology of which is briefly described below.

    The placenta is a thickened disk-shaped temporary organ that interchanges gases, nutrients, hormones, excretory products, humoral antibodies (IgG), and any other circulating substances between the maternal

21   and fetal bloodstreams. Receptors facilitate the transport of glucose, amino acids, and IgG directly from maternal blood to fetal blood. The placenta is the only organ composed of cells derived from two individuals, the fetal extraembryonic chorion and the maternal endometrium. The boundary between these two tissues is marked by extracellular products of

26   necrosis referred to as fibrinoid. This boundary results from the various tissue interactions, immunological responses, etc. which occur in the placenta.

    The major tissue interaction involves the expression of paternal antigens by the chorionic villi which is directly adjacent to maternal

31   blood. Although the mother initiates an immunological response, fetal tissue is not typically rejected. This is attributed to the fact that the fetus only expresses major histocompatibility complex (MHC) I, and not MHC II which is the major cause of organ allograft rejection. In addition, uterine secretions during early gestation contain significant amounts of

36   glucose and glycoproteins which may participate in local immunosuppression.

1   Although infections by bacteria, viruses, mycoplasmas, or parasites may
ascend from the endocervical canal or reach the placenta through maternal
blood, they rarely cause gross pathological changes because of maternal
immune defense.

6   Soon after implantation, fetal villi begin to control maternal
physiology to create an optimal environment for development. This involves
the production of chorionic gonadotropin, estrogen and progesterone,
chorionic somatomammotropin, insulin-like growth factors, platelet derived
growth factor, prolactin, and various cytokines. These and other factors
such as hjak2 certainly regulate the numerous activities (respiratory,
11   immunological, gastrointestinal, and urinary) which occur within the
placenta and between maternal and fetal tissues.

The anatomy and physiology of human placenta is reviewed, inter alia,
in Benirschke and Kaufmann, (1992) Pathology of the Human Placenta,
Springer-Verlag, New York NY, pp. 542-635; Herrera Gonzalez and Dresser
16   (1993) Dev Comp Immunol 17(1):1-18; Mitchell et al. (1993) Placenta 14:249-
275; Naeye (1992) Disorders of the Placenta, Fetus, and Neonate: Diagnosis
and Clinical Significance, Moseby Year Book, St. Louis MO; and Rutanen
(1993) Ann Med 25:343-347.

21   **SUMMARY**

The present invention relates to a novel human Jak2 kinase and to the
use of the protein and its nucleic acid acid sequence in the study,
diagnosis, prevention and treatment of diseases. Human Jak2 kinase (hjak2)
was first identified as a partial nucleotide sequence in Incyte Clone
26   179527 during a computer search for nucleotide sequence alignments among
the cDNAs of a placenta library. A modified XL-PCR procedure, specially
designed oligonucleotides, and cDNAs of the placenta library were used to
extend Incyte Clone 179527 to full length. The assembled nucleotide
sequence (SEQ ID No 1), hjak2, encodes the polypeptide (SEQ ID No 2),
31   HJAK2. Computer search and alignment of the full length amino acid
sequence showed that HJAK2 has 92% similarity to murine Jak2 kinase
(MUSPTK1; GenBank GI 409584; Wilks AF (1989) Proc Nat Acad Sci 86:1603-7)
which in turn has 96% sequence similarity with human Jak1 kinase. These
homologies and the conserved residues, G_{48}, K_{73}, E_{192}, and D_{220} which all lie
36   within the catalytic domain contributed to the naming and uses of hjak2.

                                   3

1   The complete nucleic acid sequence encoding hjak2, SEQ ID No1
disclosed herein, provides the basis for the design of antisense molecules
useful in diminishing or eliminating expression of the genomic nucleotide
sequence. For example, hjak2, or its oligonucleotides, fragments,
portions, or complement, may be used in diagnostic hybridization or

6   amplification assays of biopsied tissue to detect and/or quantify
abnormalities in gene expression associated with an immunological disorder.
The present invention also relates, in part, to the inclusion of the
nucleic acid sequence in an expression vector which can be used to
transform host cells or organisms. Such transgenic hosts are useful for

11   production and recovery of the encoded HJAK2.

The invention further comprises using purified HJAK2 polypeptide to
produce antibodies or to identify antagonists or inhibitors which bind
HJAK2. Anti-HJAK antibodies may be used in membrane, tissue-based or ELISA
technologies to detect any disease state or condition related to the

16   aberrant expression of HJAK2. Antibodies, antagonists or inhibitors can be
used to bind HJAK2 preventing the transfer of high energy phosphate
molecules and therefore signal transduction. The invention also comprises
pharmaceutical compositions containing the peptide, antibodies, antagonists
or inhibitors for the diagnosis, prevention or treatment of conditions

21   associated with altered or uncontrolled hjak2 expression. These conditions
may include, but are not limited to: arteriosclerosis, asthma, bronchitis,
emphysema, inflammatory bowel disease, leukemia, oncogenesis,
osteoarthritis, psoriasis, rheumatoid arthritis, septic shock, and systemic
lupus erythematosus. Steps for testing a biological sample with probes,

26   oligomers, fragments or portions of the hjak2 nucleotide sequence or
antibodies produced against the purified HJAK2 protein are provided.

Antisense molecules, antibodies, antagonists or inhibitors (including
proteins, peptides, oligopeptides or organic molecules capable of
compromising or modulating HJAK2 expression) may also be used for

31   therapeutic purposes, for example, in neutralizing the abberrent activity
of a HJAK2 associated with, for example, inflammation or oncogenesis. The
present invention also provides for pharmaceutical compositions for the
treatment of disease states associated with aberrant expression of hjak2
comprising the forementioned antisense molecules, antibodies, antagonists

36   or inhibitors.

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DESCRIPTION OF THE FIGURES

Figure 1 displays an alignment of the nucleic acid and amino acid sequences of human jak2 kinase. Alignments shown in this and the following figure were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 2 shows the amino acid sequence similarity between MUSPTK1, GI and HJAK2.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the abbreviation for the novel human Jak2 kinase in lower case (hjak2) refers to a gene, cDNA, RNA or nucleic acid sequence while the upper case version (HJAK2) refers to a protein, polypeptide, peptide, oligopeptide, or amino acid sequence.

An "oligonucleotide" or "oligomer" is a stretch of nucleotide residues which has a sufficient number of bases to be used in a polymerase chain reaction (PCR). These short sequences are based on (or designed from) genomic or cDNA sequences and are used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

"Probes" are nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligonucleotides. They may be single- or double-stranded and are carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

"Reporter" molecules are chemical moieties used for labelling a nucleic or amino acid sequence. They include, but are not limited to, radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents. Reporter molecules associate with, establish the presence of, and may allow quantification of a particular nucleic or amino acid sequence.

A "portion" or "fragment" of a polynucleotide or nucleic acid comprises all or any part of the nucleotide sequence having fewer nucleotides than about 6 kb, preferably fewer than about 1 kb which can be used as a probe. Such probes may be labelled with reporter molecules using nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. After pretesting to optimize reaction conditions and to eliminate false positives, nucleic acid probes may be used in Southern, northern or in situ hybridizations to determine whether DNA or RNA encoding the protein is present in a biological sample, cell type, tissue, organ or organism.

"Recombinant nucleotide variants" are polynucleotides which encode a protein. They may be synthesized by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce specific restriction sites or codon usage-specific mutations, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic host system, respectively.

"Linkers" are synthesized palindromic nucleotide sequences which create internal restriction endonuclease sites for ease of cloning the genetic material of choice into various vectors. "Polylinkers" are engineered to include multiple restriction enzyme sites and provide for the use of both those enzymes which leave 5' and 3' overhangs such as BamHI, EcoRI, PstI, KpnI and Hind III or which provide a blunt end such as EcoRV, SnaBI and StuI.

"Control elements" or "regulatory sequences" are those nontranslated regions of the gene or DNA such as enhancers, promoters, introns and 3' untranslated regions which interact with cellular proteins to carry out replication, transcription, and translation. They may occur as boundary sequences or even split the gene. They function at the molecular level and along with regulatory genes are very important in development, growth, differentiation and aging processes.

"Chimeric" molecules are polynucleotides or polypeptides which are created by combining one or more of nucleotide sequences of this invention (or their parts) with additional nucleic acid sequence(s). Such combined sequences may be introduced into an appropriate vector and expressed to

6

give rise to a chimeric polypeptide which may be expected to be different from the native molecule in one or more of the following characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signalling, etc.

"Active" refers to those forms, fragments, or domains of an amino acid sequence which display the biologic and/or immunogenic activity characteristic of the naturally occurring peptide.

"Naturally occurring HJAK2" refers to a polypeptide produced by cells which have not been genetically engineered or which have been genetically engineered to produce the same sequence as that naturally produced. Specifically contemplated are various polypeptides which arise from post-transnational modifications. Such modifications of the polypeptide include but are not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

"Derivative" refers to those polypeptides which have been chemically modified by such techniques as ubiquitination, labelling (see above), pegylation (derivatization with polyethylene glycol), and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins.

"Recombinant polypeptide variant" refers to any polypeptide which differs from naturally occurring HJAK2 by amino acid insertions, deletions and/or substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing characteristics of interest may be found by comparing the sequence of HJAK2 with that of related polypeptides and minimizing the number of amino acid sequence changes made in highly conserved regions.

Amino acid "substitutions" are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid "insertions" or "deletions" are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. The variation allowed in a particular amino acid sequence may be experimentally determined by producing the peptide synthetically or by systematically making insertions, deletions, or substitutions of

nucleotides in the hjak2 sequence using recombinant DNA techniques.

A "signal or leader sequence" is a short amino acid sequence which or can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

An "oligopeptide" is a short stretch of amino acid residues and may be expressed from an oligonucleotide. It may be functionally equivalent to and either the same length as or considerably shorter than a "fragment ", "portion ", or "segment" of a polypeptide. Such sequences comprise a stretch of amino acid residues of at least about 5 amino acids and often about 17 or more amino acids, typically at least about 9 to 13 amino acids, and of sufficient length to display biologic and/or immunogenic activity.

An "inhibitor" is a substance which retards or prevents a chemical or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives.

A "standard" is a quantitative or qualitative measurement use for comparison. Preferably, it is based on a statistically appropriate number of samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles. The samples of a particular standard may be normal or similarly abnormal.

"Animal" as used herein may be defined to include human, domestic (cats, dogs, etc), agricultural (cows, horses, sheep, goats, chicken, fish, etc) or test species (frogs, mice, rats, rabbits, simians, etc).

"Conditions" includes cancers, disorders or diseases in which hjak2 activity may be implicated. These specifically include, but are not limited to, anemia, arteriosclerosis, asthma, bronchitis, emphysema, gingivitis, inflammatory bowel disease, insulin-dependent diabetes mellitus leukemia, multiple endocrine neoplasias, osteoarthritis, osteoporosis, pulmonary fibrosis, rheumatoid arthritis, septic shock syndromes, and systemic lupus erythematosus.

Since the list of technical and scientific terms cannot be all encompassing, any undefined terms shall be construed to have the same meaning as is commonly understood by one of skill in the art to which this

1   invention belongs. Furthermore, the singular forms "a", "an" and "the"
include plural referents unless the context clearly dictates otherwise.
For example, reference to a "restriction enzyme" or a "high fidelity
enzyme" may include mixtures of such enzymes and any other enzymes fitting
the stated criteria, or reference to the method includes reference to one
6   or more methods for obtaining cDNA sequences which will be known to those
skilled in the art or will become known to them upon reading this
specification.

Before the present sequences, variants, formulations and methods for
making and using the invention are described, it is to be understood that
11   the invention is not to be limited only to the particular sequences,
variants, formulations or methods described. The sequences, variants,
formulations and methodologies may vary, and the terminology used herein is
for the purpose of describing particular embodiments. The terminology and
definitions are not intended to be limiting since the scope of protection
16   will ultimately depend upon the claims.

Description of the Invention

The present invention provides for a purified polynucleotide which
encodes a novel human Jak2 kinase which is expressed in human cells or
21   tissues. The human Jak2 kinase (hjak2; Incyte Clone 179527) was first
identified among the cDNAs from an placenta cDNA library. The naming and
proscribed uses of the present invention are based in part on the conserved
residues found in HJAK2. These particularly include the residues G_{48}, K_{73},
E_{192}, and D_{220}, which are all found within the catalytic domain. Computer
26   search and alignment of the full length amino acid sequences showed that
HJAK2 has 92% similarity to murine Jak2 kinase (MUSPTK1; GenBank GI 409584;
Wilks AF (1989) Proc Nat Acad Sci 86:1603-7) which in turn has 96% sequence
similarity with human Jak1 kinase.

Purified nucleotide sequences, such as hjak2, have numerous
31   applications in techniques known to those skilled in the art of molecular
biology. These techniques include their use as PCR or hybridization
probes, for chromosome and gene mapping, in the production of sense or
antisense nucleic acids, in screening for new therapeutic molecules, etc.
These examples are well known and are not intended to be limiting.
36   Furthermore, the nucleotide sequences disclosed herein may be used in
molecular biology techniques that have not yet been developed, provided the

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new techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair interactions.

As a result of the degeneracy of the genetic code, a multitude of HJAK2-encoding nucleotide sequences may be produced and some of these will bear only minimal homology to the endogenous sequence of any known and naturally occurring Jak2 kinase sequence. This invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HJAK2 and all such variations are to be considered as being specifically disclosed.

Although the hjak2 nucleotide sequence and its derivatives or variants are preferably capable of identifying the nucleotide sequence of the naturally occurring HJAK2 under optimized conditions, it may be advantageous to produce HJAK2-encoding nucleotide sequences possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host (REF). Other reasons for substantially altering the nucleotide sequence encoding the HJAK2 without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a longer half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding HJAK2 may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques (Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York City). Useful sequences for joining to hjak2 include an assortment of cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and the like. Vectors of interest include vectors for replication, expression, probe generation, sequencing, and the like. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for one or more host cell systems.

10

1   PCR as described in US Patent Nos. 4,683,195; 4,800,195; and
4,965,188 provides additional uses for oligonucleotides based upon the
hjak2 nucleotide sequence. Such oligomers are generally chemically
synthesized, but they may be of recombinant origin or a mixture of both.
Oligomers generally comprise two nucleotide sequences, one with sense

6   orientation (5'->3') and one with antisense (3' to 5') employed under
optimized conditions for identification of a specific gene or diagnostic
use. The same two oligomers, nested sets of oligomers, or even a
degenerate pool of oligomers may be employed under less stringent
conditions for identification and/or quantitation of closely related DNA or

11   RNA sequences.

     Full length genes may be cloned utilizing partial nucleotide sequence
and various methods known in the art. Gobinda et al (1993; PCR Methods
Applic 2:318-22) disclose "restriction-site PCR" as a direct method which
uses universal primers to retrieve unknown sequence adjacent to a known

16   locus. First, genomic DNA is amplified in the presence of primer to linker
and a primer specific to the known region. The amplified sequences are
subjected to a second round of PCR with the same linker primer and another
specific primer internal to the first one. Products of each round of PCR
are transcribed with an appropriate RNA polymerase and sequenced using

21   reverse transcriptase. Gobinda et al present data concerning Factor IX for
which they identified a conserved stretch of 20 nucleotides in the 3'
noncoding region of the gene.

     Inverse PCR is the first method to report successful acquisition of
unknown sequences starting with primers based on a known region (Triglia T

26   et al(1988) Nucleic Acids Res 16:8186). The method uses several restriction
enzymes to generate a suitable fragment in the known region of a gene. The
fragment is then circularized by intramolecular ligation and used as a PCR
template. Divergent primers are designed from the known region. The
multiple rounds of restriction enzyme digestions and ligations that are

31   necessary prior to PCR make the procedure slow and expensive (Gobinda et
al, supra).

     Capture PCR (Lagerstrom M et al (1991) PCR Methods Applic 1:111-19)
is a method for PCR amplification of DNA fragments adjacent to a known
sequence in human and YAC DNA. As noted by Gobinda et al (supra), capture

36   PCR also requires multiple restriction enzyme digestions and ligations to

11

1   place an engineered double-stranded sequence into an unknown portion of the
     DNA molecule before PCR. Although the restriction and ligation reactions
     are carried out simultaneously, the requirements for extension,
     immobilization and two rounds of PCR and purification prior to sequencing
     render the method cumbersome and time consuming.

6   Parker JD et al (1991; Nucleic Acids Res 19:3055-60), teach walking
     PCR, a method for targeted gene walking which permits retrieval of unknown
     sequence. In this same vein, PromoterFinder™ a new kit available from
     Clontech (Palo Alto CA) uses PCR and primers derived from p53 to walk in
     genomic DNA. Nested primers and special PromoterFinder libraries are used
11   to detect upstream sequences such as promoters and regulatory elements.
     This process avoids the need to screen libraries and is useful in finding
     intron/exon junctions.

          Another new PCR method, "Improved Method for Obtaining Full Length
     cDNA Sequences" by Guegler et al, Patent Application Serial No 08/487,112,
16   filed June 7, 1995 and hereby incorporated by reference, employs XL-PCR™
     (Perkin-Elmer, Foster City CA) to amplify and extend partial nucleotide
     sequence into longer pieces of DNA. This method was developed to allow a
     single researcher to process multiple genes (up to 20 or more) at one time
     and to obtain an extended (possibly full-length) sequence within 6-10 days.
21   This new method replaces methods which use labelled probes to screen
     plasmid libraries and allow one researcher to process only about 3-5 genes
     in 14-40 days.

          In the first step, which can be performed in about two days, any two
     of a plurality of primers are designed and synthesized based on a known
26   partial sequence. In step 2, which takes about six to eight hours, the
     sequence is extended by PCR amplification of a selected library. Steps 3
     and 4, which take about one day, are purification of the amplified cDNA and
     its ligation into an appropriate vector. Step 5, which takes about one
     day, involves transforming and growing up host bacteria. In step 6, which
31   takes approximately five hours, PCR is used to screen bacterial clones for
     extended sequence. The final steps, which take about one day, involve the
     preparation and sequencing of selected clones.

          If the full length cDNA has not been obtained, the entire procedure
     is repeated using either the original library or some other preferred
36   library. The preferred library may be one that has been size-selected to

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include only larger cDNAs or may consist of single or combined commercially available libraries, eg. lung, liver, heart and brain from Gibco/BRL (Gaithersburg MD). The cDNA library may have been prepared with oligo (dT) or random priming. Random primed libraries are preferred in that they will contain more sequences which contain 5' ends of genes. A randomly primed library may be particularly useful if an oligo (dT) library does not yield a complete gene. It must be noted that the larger and more complex the protein, the less likely it is that the complete gene will be found in a single plasmid.

A new method for analyzing either the size or the nucleotide sequence of PCR products is capillary electrophoresis. Systems for rapid sequencing are available from Perkin Elmer (Foster City CA), Beckman Instruments (Fullerton CA), and other companies. Capillary sequencing employs flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity is converted to electrical signal using appropriate software (eg. Genotyper™ and Sequence Navigator™ from Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display is computer controlled. Capillary electrophoresis provides greater resolution and is many times faster than standard gel based procedures. It is particularly suited to the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample. The reproducible sequencing of up to 350 bp of M13 phage DNA in 30 min has been reported (Ruiz-Martinez MC et al (1993) Anal Chem 65:2851-8).

Another aspect of the subject invention is to provide for hjak2 hybridization probes which are capable of hybridizing with naturally occurring nucleotide sequences encoding HJAK2. The stringency of the hybridization conditions will determine whether the probe identifies only the native nucleotide sequence of hjak2 or sequences of other closely related Jak2 kinase molecules. If degenerate hjak2 nucleotide sequences of the subject invention are used for the detection of related kinase encoding sequences, they should preferably contain at least 50% of the nucleotides of the sequences presented herein. Hybridization probes of the subject invention may be derived from the nucleotide sequence presented in SEQ ID NO 1 or from surrounding genomic sequences comprising untranslated regions

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such as promoters, enhancers and introns. Such hybridization probes may be labelled with appropriate reporter molecules.

Means for producing specific hybridization probes for this Jak2 kinase include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled nucleotide. Alternatively, the cDNA sequence may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes _in_ _vitro_ by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. A number of companies (such as Pharmacia Biotech, Piscataway NJ; Promega, Madison WI; US Biochemical Corp, Cleveland, OH; etc.) supply commercial kits and protocols for these procedures.

It is also possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. Sometimes the source of information for producing this sequence comes from the known homologous sequence from closely related organisms. After synthesis, the nucleic acid sequence can be used alone or joined with a pre-existing sequence and inserted into one of the many available DNA vectors and their respective host cells using techniques well known in the art. Moreover, synthetic chemistry may be used to introduce specific mutations into the nucleotide sequence. Alternatively, a portion of sequence in which a mutation is desired can be synthesized and recombined with a portion of an existing genomic or recombinant sequence.

Hjak2 nucleotide sequence can be used in a diagnostic test or assay to detect disorder or disease processes associated with abnormal expression of hjak2. The nucleotide sequence is added to a sample (fluid, cell or tissue) from a patient under hybridizing conditions. After an incubation period, the sample is washed with a compatible fluid which optionally contains a reporter molecule which will bind the specific nucleotide. After the compatible fluid is rinsed off, the reporter molecule is quantitated and compared with a standard for that fluid, cell or tissue. If hjak2 expression is significantly different from the standard, the assay indicates the presence of disorder or disease. The form of such qualitative or quantitative methods may include northern analysis, dot blot or other membrane-based technologies, dip stick, pin or chip technologies, PCR, ELISAs or other multiple sample format technologies.

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1   This same assay, combining a sample with the nucleotide sequence, is
applicable in evaluating the efficacy of a particular therapeutic treatment
regime. It may be used in animal studies, in clinical trials, or in
monitoring the treatment of an individual patient. First, standard
expression must be established for use as a basis of comparison. Second,
6   samples from the animals or patients affected by a disorder or disease are
combined with the nucleotide sequence to evaluate the deviation from the
standard or normal profile. Third, an entirely new or pre-existing
therapeutic agent is administered, and a treatment profile is generated.
This posat-treatment assay is evaluated to determine whether the patient
11   profile progresses toward or returns to the standard pattern. Successive
treatment profiles may be used to show the efficacy of treatment over a
period of several days or several months.

        The nucleotide sequence for hjak2 can also be used to generate probes
for mapping native genomic sequence. The sequence may be mapped to a
16   particular chromosome or to a specific region of the chromosome using well
known techniques. These include in situ hybridization to chromosomal
spreads (Verma et al (1988) Human Chromosomes: A Manual of Basic
Techniques, Pergamon Press, New York City), flow-sorted chromosomal
preparations, or artificial chromosome constructions such as yeast
21   artificial chromosomes (YACs), bacterial artificial chromosomes (BACs),
bacterial P1 constructions or single chromosome cDNA libraries.

        In situ hybridization of chromosomal preparations and physical
mapping techniques such as linkage analysis using established chromosomal
markers are invaluable in extending genetic maps. Examples of such genetic
26   maps can regularly be found in the journal Science (eg, 1994; 265:1981f).
Often the placement of a gene on the chromosome of another mammalian
species may reveal associated markers even if the number or arm of a
particular human chromosome is not known. New sequences can be assigned to
chromosomal arms, or parts thereof, by physical mapping. This provides
31   valuable information to investigators searching for disease genes using
positional cloning or other gene discovery techniques. Once a disease or
syndrome, such as ataxia telangiectasia (AT), has been crudely localized by
genetic linkage to a particular genomic region, for example, AT to 11q22-23
(Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area
36   may represent associated or regulatory genes for further investigation.

15

1   The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. between normal and carrier or affected individuals.

The nucleotide sequence encoding HJAK2 may be used to produce an amino acid sequence using well known methods of recombinant DNA technology.

6   Goeddel (1990, Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego CA) is one among many publications which teach expression of an isolated, purified nucleotide sequence. The amino acid or peptide may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which the

11   nucleotide sequence was derived or from a different species. Advantages of producing an amino acid sequence or peptide by recombinant DNA technology include obtaining adequate amounts for purification and the availability of simplified purification procedures.

Cells transformed with hjak2 nucleotide sequence may be cultured

16   under conditions suitable for the expression and recovery of peptide from cell culture. The peptide produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. In general, it is more convenient to prepare recombinant proteins in secreted form, and this is accomplished by ligating hjak2 to a

21   recombinant nucleotide sequence which directs its movement through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join hjak2 to nucleotide sequence encoding a polypeptide domain which will facilitate protein purification (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

26   Direct peptide synthesis using solid-phase techniques (Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY pp. 50-60) is an alternative to recombinant or chimeric peptide production. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer in accordance with the

31   instructions provided by the manufacturer. Additionally HJAK2 or any part thereof may be mutated during direct synthesis and combined using chemical methods with other kinase sequences, or parts thereof.

Although an amino acid sequence or oligopeptide used for antibody induction does not require biological activity, it must be immunogenic.

36   HJAK2 used to induce specific antibodies may have an amino acid sequence

1   consisting of at least five amino acids and preferably at least 10 amino
acids. Short stretches of amino acid sequence may be fused with those of
another protein such as keyhole limpet hemocyanin, and the chimeric peptide
used for antibody production. Alternatively, the peptide may be of
sufficient length to contain an entire domain.

6   Antibodies specific for HJAK2 may be produced by inoculation of an
appropriate animal with an antigenic fragment of the peptide. An antibody
is specific for HJAK2 if it is produced against an epitope of the
polypeptide and binds to at least part of the natural or recombinant
protein.   Antibody production includes not only the stimulation of an
11   immune response by injection into animals, but also analogous processes
such as the production of synthetic antibodies, the screening of
recombinant immunoglobulin libraries for specific-binding molecules
(Orlandi R et al (1989) PNAS 86:3833-3837, or Huse WD et al (1989) Science
256:1275-1281), or the in vitro stimulation of lymphocyte populations.
16   Current technology (Winter G and Milstein C (1991) Nature 349:293-299)
provides for a number of highly specific binding reagents based on the
principles of antibody formation. These techniques may be adapted to
produce molecules which specifically bind HJAK2. Antibodies or other
appropriate molecules generated against a specific immunogenic peptide
21   fragment or oligopeptide can be used in Western analysis, enzyme-linked
immunosorbent assays (ELISA) or similar tests to establish the presence of
or to quantitate amounts of HJAK2 active in normal, diseased, or
therapeutically treated cells or tissues.

The examples below are provided to illustrate the subject invention.
26   These examples are provided by way of illustration and are not included for
the purpose of limiting the invention.

EXAMPLES

31   I   **Placenta cDNA Library Construction**
Library was constructed from normal placenta obtained from the Mayo
Clinic. The tissue was lysed in a buffer containing guanidinium
isothiocyanate. The lysate was extracted with phenol chloroform and
precipitated with ethanol. Poly A⁺ RNA was isolated using biotinylated
36   oligo d(T) primer and steptavidin coupled to a paramagnetic particle

(Promega Corp. Madison WI) and sent to Stratagene (La Jolla CA) for cDNA library preparation. The cDNA synthesis was primed using both oligo d(T) and random hexamers, and the two cDNA libraries were treated separately. Synthetic adapter oligonucleotides were ligated onto the ends of the cDNAs which were digested with XhoI and inserted into the Uni-ZAP™ vector system (Stratagene).

The pBluescript™ phagemid (Stratagene) was excised from each library , and phagemids from the two cDNA libraries were combined into a single library by mixing equal numbers of bacteriophage. The phagemids were transformed into E. coli host strain XL1-Blue™ (Stratagene). Enzymes from both pBluescript and a cotransformed f1 helper phage nicked the DNA, initiated new DNA synthesis, and created the smaller, single-stranded circular plasmid DNA molecules which contained the cDNA insert. The plasmid DNA was released, purified, and used to reinfect fresh host cells (SOLR, Stratagene). Presence of the ß-lactamase gene on the plasmid allowed transformed bacteria to grow on medium containing ampicillin.

II   Isolation of cDNA Clones

Plasmid DNAs containing the cDNA insert were purified using the QIAWELL-8 Plasmid Purification System from QIAGEN Inc (Chatsworth CA) according to standard protocol. The DNA was eluted and prepared for DNA sequencing and other analytical manipulations.

The cDNA inserts from random isolates of the placenta library were partially sequenced. The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Catalyst 800 or a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer), and reading frame was determined.

III   Sequencing of cDNA Clones

The cDNA inserts from random isolates of the placenta library were sequenced in part. Methods for DNA sequencing are well known in the art and employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE® (US Biochemical Corp) or Taq polymerase. Methods to extend the DNA from an oligonucleotide primer annealed to the DNA template of interest have been developed for both single- and double-stranded templates. Chain

1   termination reaction products were separated using electrophoresis and
       detected via their incorporated, labelled precursors. Recent improvements
       in mechanized reaction preparation, sequencing and analysis have permitted
       expansion in the number of sequences that can be determined per day.
       Preferably, the process is automated with machines such as the Hamilton
6   Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ
       Research, Watertown MA) and the Applied Biosystems Catalyst 800 and 377 and
       373 DNA sequencers.

            The quality of any particular cDNA library may be determined by
       performing a pilot scale analysis of the cDNAs and checking for percentages
11   of clones containing vector, lambda or E. coli DNA, mitochondrial or
       repetitive DNA, and clones with exact or homologous matches to public
       databases. The number of unique sequences, those having no known match in
       any available database, are then recorded.

16   **IV   Homology Searching of cDNA Clones and Their Deduced Proteins**

            Each sequence so obtained was compared to sequences in GenBank using
       a search algorithm developed by Applied Biosystems and incorporated into
       the INHERIT" 670 Sequence Analysis System. In this algorithm, Pattern
       Specification Language (TRW Inc, Los Angeles CA) was used to determine
21   regions of homology. The three parameters that determine how the sequence
       comparisons run were window size, window offset, and error tolerance.
       Using a combination of these three parameters, the DNA database was
       searched for sequences containing regions of homology to the query
       sequence, and the appropriate sequences were scored with an initial value.
26   Subsequently, these homologous regions were examined using dot matrix
       homology plots to distinguish regions of homology from chance matches.
       Smith-Waterman alignments were used to display the results of the homology
       search.

            Peptide and protein sequence homologies were ascertained using the
31   INHERIT" 670 Sequence Analysis System in a way similar to that used in DNA
       sequence homologies. Pattern Specification Language and parameter windows
       were used to search protein databases for sequences containing regions of
       homology which were scored with an initial value. Dot-matrix homology
       plots were examined to distinguish regions of significant homology from
36   chance matches.

            Alternatively, BLAST, which stands for Basic Local Alignment Search

1 Tool, is used to search for local sequence alignments (Altschul SF (1993) J
Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10).
BLAST produces alignments of both nucleotide and amino acid sequences to
determine sequence similarity. Because of the local nature of the
alignments, BLAST is especially useful in determining exact matches or in
6 identifying homologs. While it is useful for matches which do not contain
gaps, it is inappropriate for performing motif-style searching. The
fundamental unit of BLAST algorithm output is the High-scoring Segment Pair
(HSP).

An HSP consists of two sequence fragments of arbitrary but equal
11 lengths whose alignment is locally maximal and for which the alignment
score meets or exceeds a threshold or cutoff score set by the user. The
BLAST approach is to look for HSPs between a query sequence and a database
sequence, to evaluate the statistical significance of any matches found,
and to report only those matches which satisfy the user-selected threshold
16 of significance. The parameter E establishes the statistically significant
threshold for reporting database sequence matches. E is interpreted as
the upper bound of the expected frequency of chance occurrence of an HSP
(or set of HSPs) within the context of the entire database search. Any
database sequence whose match satisfies E is reported in the program
21 output.

The partial hjak2 molecule presented and claimed in this application
was identified using the criteria above. The full length nucleic and amino
acid sequences for this novel human Jak2 kinase are shown in Fig 1. Fig 2
shows the alignment between the translated amino acid sequence for hjak2
26 and the closest related molecule, murine Jak2 kinase (MUSPTK1; GenBank GI
409584; Wilks AF (1989) Proc Nat Acad Sci 86:1603-7).

V   **Extension of cDNAs to Full Length**

The partial sequence originally identified in Incyte Clone 179527 was
31 used to design oligonucleotide primers for extension of the cDNAs to full
length. Primers are designed based on known sequence; one primer is
synthesized to initiate extension in the antisense direction (XLR) and the
other to extend sequence in the sense direction (XLF). The primers allow
the sequence to be extended "outward" generating amplicons containing new,
36 unknown nucleotide sequence for the gene of interest. The primers may be
designed using Oligo 4.0 (National Biosciences Inc, Plymouth MN), or

another appropriate program, to be 22-30 nucleotides in length, to have a
GC content of 50% or more, and to anneal to the target sequence at
temperatures about 68°-72° C.   Any stretch of nucleotides which would
result in hairpin structures and primer-primer dimerizations was avoided.

The placenta cDNA library was used with XLR =
GGGCGGAAGTGCTCTCGGCGGAAG and XLF = AGTGTGCTACAGTGCTGGTCGTCG primers to
extend and amplify Incyte Clone 179527 to obtain the full length Jak2
kinase sequence.

By following the instructions for the XL-PCR kit and thoroughly
mixing the enzyme and reaction mix, high fidelity amplification is
obtained.   Beginning with 40 pmol of each primer and the recommended
concentrations of all other components of the kit, PCR is performed using
the Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the
following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5-10 μl aliquot of the reaction mixture is analyzed by
electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to
determine which reactions were successful in extending the sequence.
Although all extensions potentially contain a full length gene, some of the
largest products or bands are selected and cut out of the gel.   Further
purification involves using a commercial gel extraction method such as
QIAQuick™ (QIAGEN Inc).   After recovery of the DNA, Klenow enzyme is used
to trim single-stranded, nucleotide overhangs creating blunt ends which
facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μl of
ligation buffer.   Then, 1μl T4-DNA ligase (15 units) and 1μl T4
polynucleotide kinase are added, and the mixture is incubated at room
temperature for 2-3 hours or overnight at 16° C.   Competent E. coli cells

21

(in 40 μl of appropriate media) are transformed with 3 μl of ligation
mixture and cultured in 80 μl of SOC medium (Sambrook J et al, supra).
After incubation for one hour at 37° C, the whole transformation mixture is
plated on Luria Bertani (LB)-agar (Sambrook J et al, supra) containing
2xCarb. The following day, 12 colonies are randomly picked from each plate
and cultured in 150 μl of liquid LB/2xCarb medium placed in an individual
well of an appropriate, commercially-available, sterile 96-well microtiter
plate. The following day, 5 μl of each overnight culture is transferred
into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μl
of each sample is transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x)
containing 4 units of rTth DNA polymerase, a vector primer and one or both
of the gene specific primers used for the extension reaction are added to
each well. Amplification is performed using the following conditions:

Step 1   94° C for 60 sec
Step 2   94° C for 20 sec
Step 3   55° C for 30 sec
Step 4   72° C for 90 sec
Step 5   - Repeat steps 2-4 for an additional 29 cycles
Step 6   72° C for 180 sec
Step 7   4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with
molecular weight markers. The sizes of the PCR products are compared to
the original partial cDNAs, and appropriate clones are selected, ligated
into plasmid and sequenced.

VI   Diagnostic Assay Using Hjak2 Specific Oligomers

In those cases where a specific condition (see definitions, supra) is
suspected to involve expression of altered quantities of hjak2, oligomers
may be designed to establish the presence and/or quantity of mRNA expressed
in a biological sample. There are several methods currently being used to
quantitate the expression of a particular molecule. Most of these methods
use radiolabelled (Melby PC et al 1993 J Immunol Methods 159:235-44) or
biotinylated (Duplaa C et al 1993 Anal Biochem 229-36) nucleotides,
coamplification of a control nucleic acid, and standard curves onto which
the experimental results are interpolated. Quantitation may be speeded up
by running the assay in an ELISA format where the oligomer-of-interest is
presented in various dilutions and a colorimetric response gives rapid

22

1   quantitation. For example, a complete HJAK2 deficiency may result in the
     inability to undergo cell division or to react to an infectious organism.
     In like manner, overexpression may cause major inflammation, swelling and
     major tissue damage. In either case, a quick diagnosis may allow health
     professionals to treat the condition and prevent worsening of the
6   condition. This same assay can be used to monitor progress of the patient
     as his/her physiological situation moves toward the normal range during
     therapy.

                                                      .

     VII   Sense or Antisense Molecules
11   Knowledge of the correct cDNA sequence of this Jak2 kinase or its
     regulatory elements enable its use as a tool in sense (Youssoufian H and HF
     Lodish 1993) Mol Cell Biol 13:98-104) or antisense (Eguchi et al (1991)
     Annu Rev Biochem 60:631-652) technologies for the investigation or
     alteration of gene expression. To inhibit in vivo or in vitro cdp
16   expression, an oligonucleotide based on the coding sequence of an hjak2
     designed with Oligo 4.0 (National Biosciences Inc) is used. Alternatively,
     a fragment of an hjak2 is produced by digesting hjak2 coding sequence with
     restriction enzymes. These enzymes and specific restrictions sites may be
     selected using Inherit Analysis software (Applied Biosystems), and the
21   strands separated by heating the fragments and selecting for the antisense
     strand. Either the oligonucleotide or the fragment may be used to inhibit
     hjak2 expression. Furthermore, antisense molecules can be designed to
     inhibit promoter binding in the upstream nontranslated leader or at various
     sites along the hjak2 coding region. Alternatively, antisense molecules
26   may be designed to inhibit translation of an mRNA into polypeptide by
     preparing an oligomer or fragment which will bind in the region spanning
     approximately -10 to +10 nucleotides at the 5' end of the coding sequence.
     These technologies are now well known to those of in the art.
             In addition to using antisense molecules constructed to interrupt
31   transcription of the open reading frame, modifications of gene expression
     can be obtained by designing antisense sequences to enhancers, introns, or
     even to trans-acting regulatory genes. Similarly, inhibition can be
     achieved using Hogeboom base-pairing methodology, also known as "triple
     helix" base pairing. Triple helix pairing compromises the ability of the
36   double helix to open sufficiently for the binding of polymerases,

                                      23

1   transcription factors, or regulatory molecules.

Any of these types of antisense molecules may be placed in expression vectors and used to transform preferred cells or tissues. This may include introduction of the expression vector into a organ, tumor, synovial cavity or the vascular system for transient or short term therapy or

6   introduction via gene therapy technologies for long term treatment. Transient expression may last for a month or more with a non-replicating vector and three months or more if appropriate replication elements are used in the transformation or expression system.

Stable transformation of appropriate dividing cells with a vector

11   containing the antisense molecule can produce a transgenic cell line, tissue or organism (see, for example, Trends in Biotechnol 11:155-215 (1993) and US Patent No. 4,736,866, 12 April 1988). Those cells which assimilate or replicate enough copies of the vector to allow stable integration will also produce enough antisense molecules to compromise or

16   entirely eliminate normal activity of the hjak2. Frequently, the function of an hjak2 can be ascertained by observing behaviors such as lethality, loss of a physiological pathway, changes in morphology, etc. at the cellular, tissue or organismal level.

21   VIII   **Expression of HJAK2**

Expression of the HJAK2 may be accomplished by subcloning the cDNA into appropriate vectors and transfecting the vectors into host cells. In this case, the cloning vector previously used for the generation of the tissue library also provides for direct expression of the hjak2 sequence in

26   E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

31   Induction of an isolated, transfected bacterial strain with IPTG using standard methods will produce a fusion protein corresponding to the first seven residues of ß-galactosidase, about 5 to 15 residues which correspond to linker, and the peptide encoded within the hjak2 cDNA. Since cDNA clone inserts are generated by an essentially random process, there is

36   one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it

1   can be obtained by deletion or insertion of the appropriate number of bases
by well known methods including in vitro mutagenesis, digestion with
exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for
6   expression of protein in specific hosts. Oligonucleotide linkers
containing cloning sites as well as a stretch of DNA sufficient to
hybridize to the end of the target cDNA (25 bases) can be synthesized
chemically by standard methods. These primers can then used to amplify the
desired gene fragments by PCR. The resulting fragments can be digested
11   with appropriate restriction enzymes under standard conditions and isolated
by gel electrophoresis. Alternatively, similar gene fragments can be
produced by digestion of the cDNA with appropriate restriction enzymes and
filling in the missing gene sequence with chemically synthesized
oligonucleotides. Partial nucleotide sequence from more than one kinase
16   homolog can be ligated together and cloned into appropriate vectors to
optimize expression.

Suitable expression hosts for such chimeric molecules include but are
not limited to mammalian cells such as Chinese Hamster Ovary (CHO) and
human 293 cells, insect cells such as Sf9 cells, yeast cells such as
21   Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these
cell systems, a useful expression vector may also include an origin of
replication to allow propagation in bacteria and a selectable marker such
as the ß-lactamase antibiotic resistance gene to allow selection in
bacteria. In addition, the vectors may include a second selectable marker
26   such as the neomycin phosphotransferase gene to allow selection in
transfected eukaryotic host cells. Vectors for use in eukaryotic
expression hosts may require RNA processing elements such as 3'
polyadenylation sequences if such are not part of the cDNA of interest.

If native promoters are not part of the cDNA, other host specific
31   promoters may be specifically combined with the coding region of hjak2.
They include MMTV, SV40, and metallothionine promoters for CHO cells; trp,
lac, tac and T7 promoters for bacterial hosts; and alpha factor, alcohol
oxidase and PGH promoters for yeast. In addition, transcription enhancers,
such as the rous sarcoma virus (RSV) enhancer, may be used in mammalian
36   host cells. Once homogeneous cultures of recombinant cells are obtained

1   through standard culture methods, large quantities of recombinantly
produced peptide can be recovered from the conditioned medium and analyzed
using methods known in the art.

IX   Isolation of Recombinant HJAK2

6   HJAK2 may be expressed as a recombinant protein with one or more
additional polypeptide domains added to facilitate protein purification.
Such purification facilitating domains include, but are not limited to,
metal chelating peptides such as histidine- tryptophan modules that allow
purification on immobilized metals, protein A domains that allow.

11   purification on immobilized immunoglobulin, and the domain utilized in the
FLAGS extension/affinity purification system (Immunex Corp, Seattle WA).
The inclusion of a cleavable linker sequence such as Factor XA or
enterokinase (Invitrogen) between the purification domain and the hjak2
sequence may be useful to facilitate expression of HJAK2.

X   Testing HJAK2 Activity

     The sequence for HJAK2 in this application present many different
domains (and subdomains as detailed in the background of the invention)
which may be utilized: 1) individually for the production of antibodies, 2)

21   in functional groups (eg. to span a membrane), and 3) as interchangable,
usable parts of a chimeric kinase. For example, a known, full length
kinase such as the hjak2 kinase of this application may be used to swap
related portions of the nucleic acid sequence, analogous to domains or
subdomains of MAP kinase polypeptides. The chimeric nucleotides, so

26   produced, may be introduced into prokaryotic host cells (as reviewed in
Strosberg AD and Marullo S (1992) Trends Pharma Sci 13:95-98) or eukaryotic
host cells. These host cells are then employed in procedures to determine
what molecules activate the kinase or what molecules are activated by a
kinase. Such activating or activated molecules may be of extracellular,

31   intracellular, biologic or chemical origin.

     An example of a test system, in this case for hjak2 kinase, can be
based on the interaction of protein tyrosine kinases with chemokine
receptors (Taniguchi T (1995) Science 268:251-255). These receptors are
capable of activating a variety of nonreceptor protein tyrosine kinases

36   when stimulated by an extracellular chemokine. C-X-C chemokines such as

1   platelet factor 4, interleukin-8, connective tissue activating protein III,
neutrophil activating peptide 2, are soluble activators of neutrophils.

A standard measure of neutrophil activation involves measuring the
mobilization of Ca^{++} as part of the signal transduction pathway. The
experiment involves several steps. First, blood cells obtained from

6   venipuncture are fractionated by centrifugation on density gradients.
Enriched populations of neutrophils are further fractionated on columns by
negative selection using antibodies specific for other blood cells types.
Next, neutrophils are transformed with an expression vector containing the
kinase nucleic acid sequence of interest and preloaded fluorescent probe

11   whose emission characteristics have been altered by Ca^{++} binding. Or in
the alternative, the neutrophil is preloaded with the purified kinase of
interest and fluorescent probe. Then, when the cells are exposed to an
appropriate chemokine, the chemokine receptor activates the kinase which,
in turn, initiates Ca^{++} flux. Ca^{++} mobilization is observed and measured

16   using fluorometry as has been described in Grynkievicz G et al (1985) J
Biol Chem 260:3440, and McColl S et al (1993) J Immunol 150:4550-4555,
incorporated herein by reference.

XI   Identification of or Production of HJAK2 Specific Antibodies

21   Purified HJAK2 is used to screen a pre-existing antibody library or
to raise antibodies using either polyclonal or monoclonal methodology. In
a polyclonal approach, denatured protein from the reverse phase HPLC
separation is obtained in quantities up to 75 mg. This denatured protein
can be used to immunize mice or rabbits using standard protocols; about 100

26   micrograms are adequate for immunization of a mouse, while up to 1 mg might
be used to immunize a rabbit. For identifying mouse hybridomas, the
denatured protein can be radioiodinated and used to screen potential murine
B-cell hybridomas for those which produce antibody. This procedure
requires only small quantities of protein, such that 20 mg would be

31   sufficient for labeling and screening of several thousand clones.

In a monoclonal approach, the amino acid sequence of HJAK2, as
deduced from translation of the cDNA, is analyzed to determine regions of
high immunogenicity. Oligopeptides comprising appropriate hydrophilic
regions, as shown in Fig. 3, are synthesized and used in suitable

36   immunization protocols to raise antibodies. Analysis to select appropriate
epitopes is described by Ausubel FM et al (supra). The optimal amino acid

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1   sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

6   Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl- N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine may be introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are

11   immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% BSA, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

16   Hybridomas may also be prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled HJAK2 to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated with affinity purified, specific

21   rabbit-anti-mouse antibodies (or suitable anti-species Ig) at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and exposed to supernatants from hybridomas. After incubation the wells are exposed to labeled HJAK2, 1 mg/ml. Clones producing antibodies will bind a quantity of labeled HJAK2 which is detectable above background. Such clones are

26   expanded and subjected to 2 cycles of cloning at limiting dilution (1 cell/3 wells). Cloned hybridomas are injected into pristine mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least 10^8 /M, preferably 10^9 to 10^{10} or stronger, will

31   typically be made by standard procedures as described in Harlow and Lane (1988) <u>Antibodies: A Laboratory Manual</u>, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; and in Goding (1986) <u>Monoclonal Antibodies: Principles and Practice</u>, Academic Press, New York City, both incorporated herein by reference.

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XII Diagnostic Test Using HJAK2 Specific Antibodies

Particular HJAK2 antibodies are useful for the diagnosis of prepathologic conditions, and chronic or acute diseases which are characterized by differences in the amount or distribution of HJAK2. To date, HJAK2 has been found only in the placenta library; however, its activity there is most probably associated with organ function, inflammation or defense.

Diagnostic tests for HJAK2 include methods utilizing the antibody and a label to detect HJAK2 in human body fluids, tissues or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemi-luminescent, or chromogenic agents previously mentioned as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567, incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound HJAK2, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HJAK2 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

XIII   Purification of Native HJAK2 Using Specific Antibodies

Native or recombinant HJAK2 can be purified by immunoaffinity chromatography using antibodies specific for that particular HJAK2. In general, an immunoaffinity column is constructed by covalently coupling the

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1   anti-HJAK2 antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia Biotech). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or

6   chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia Biotech). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

11   Such immunoaffinity columns may be utilized in the purification of HJAK2 by preparing a fraction from cells containing HJAK2 in a soluble form. This preparation may be derived by solubilization of whole cells or of a subcellular fraction obtained via differential centrifugation (with or without addition of detergent) or by other methods well known in the art. 16   Alternatively, soluble HJAK2 containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble HJAK2-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HJAK2 (eg, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions 21   that disrupt antibody/HJAK2 binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and HJAK2 is collected.

26   **XIV   Drug Screening**

This invention is particularly useful for screening therapeutic compounds by using binding fragments of HJAK2 in any of a variety of drug screening techniques. The peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell 31   surface or located intracellularly. One may measure, for example, the formation of complexes between HJAK2 and the agent being tested. Alternatively, one can examine the diminution in complex formation between HJAK2 and a receptor caused by the agent being tested.

Methods of screening for drugs or any other agents which can affect 36   macrophage activation comprise contacting such an agent with HJAK2 fragment and assaying for the presence of a complex between the agent and the HJAK2

30

1   fragment. In such assays, the HJAK2 fragment is typically labelled. After

suitable incubation, free HJAK2 fragment is separated from that present in

bound form, and the amount of free or uncomplexed label is a measure of the

ability of the particular agent to bind to HJAK2.

Another technique for drug screening provides high throughput

6   screening for compounds having suitable binding affinity to the HJAK2

polypeptides and is described in detail in European Patent Application

84/03564, published on September 13, 1984, incorporated herein by

reference. Briefly stated, large numbers of different small peptide test

compounds are synthesized on a solid substrate, such as plastic pins or

11   some other surface. The peptide test compounds are reacted with HJAK2

fragment and washed. Bound HJAK2 fragment is then detected by methods well

known in the art. Purified HJAK2 can also be coated directly onto plates

for use in the aforementioned drug screening techniques. In addition, non-

neutralizing antibodies can be used to capture the peptide and immobilize

16   it on the solid support.

This invention also contemplates the use of competitive drug

screening assays in which neutralizing antibodies capable of binding HJAK2

specifically compete with a test compound for binding to HJAK2 fragments.

In this manner, the antibodies can be used to detect the presence of any

21   peptide which shares one or more antigenic determinants with HJAK2.

XV   Identification of Molecules Which Interact with HJAK2

The inventive purified HJAK2 is a research tool for identification,

characterization and purification of interacting molecules. Appropriate

26   labels are incorporated into HJAK2 by various methods known in the art and

HJAK2 is used to capture soluble or interact with membrane-bound molecules.

A preferred method involves labeling the primary amino groups in HJAK2

with [125]I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J

133: 529). This reagent has been used to label various molecules without

31   concomitant loss of biological activity (Hebert CA et al (1991) J Biol Chem

266: 18989-94; McColl S et al (1993) J Immunol 150:4550-4555). Membrane-

bound molecules are incubated with the labelled HJAK2 molecules, washed to

removed unbound molecules, and the HJAK2 complex is quantified. Data

obtained using different concentrations of HJAK2 are used to calculate

36   values for the number, affinity, and association of HJAK2.

Labelled HJAK2 fragments are also useful as a reagent for the

31

1   purification of molecules with which HJAK2 interacts, specifically
     including inhibitors. In one embodiment of affinity purification, HJAK2 is
     covalently coupled to a chromatography column. Cells and their membranes
     are extracted, HJAK2 is removed and various HJAK2-free subcomponents are
     passed over the column. Molecules bind to the column by virtue of their
6   HJAK2 affinity. The HJAK2-complex is recovered from the column,
     dissociated and the recovered molecule is subjected to N-terminal protein
     sequencing or other identification procedure. If the captured molecule has
     an amino acid sequence, it can be used to design degenerate oligomers for
     use in cloning the gene from an appropriate cDNA library.

11   In an alternate method, monoclonal antibodies raised against HJAK2
     fragments are screened to identify those which inhibit the binding of
     labelled HJAK2. These monoclonal antibodies are then used in affinity
     purification or expression cloning of associated molecules. Other soluble
     binding molecules are identified in a similar manner. Labelled HJAK2 is
16   incubated with extracts or other appropriate materials derived from lung,
     kidney or other tissues with activated monocytes or macrophages. After
     incubation, HJAK2 complexes (which are larger than the lone HJAK2 fragment)
     are identified by a sizing technique such as size exclusion chromatography
     or density gradient centrifugation and are purified by methods well known
21   in the art. The soluble binding protein(s) are subjected to N-terminal
     sequencing to obtain information sufficient for database identification, if
     the soluble protein is known, or for cloning, if the soluble protein is
     unknown.

26   XVI   Use and Administration of Antibodies or Inhibitors to HJAK2
         The antibodies and inhibitors can provide different effects when
     administered therapeutically. The antibodies and inhibitors are used to
     lessen or eliminate undue damage caused by disorders or diseases associated
     with upregulated HJAK2 expression. Each of these molecules or treatments
31   (TSTs) will be formulated in a nontoxic, inert, pharmaceutically acceptable
     aqueous carrier medium preferably at a pH of about 5 to 8, more preferably
     6 to 8, although the pH may vary according to the different characteristics
     of the peptide, antibody or inhibitor being formulated and the condition to
     be treated. Characteristics of TSTs include solubility of the molecule,
36   half-life, antigenicity/immunogenicity and the ability of the inhibitor to
     reach its target(s). These and other characteristics may aid in defining

                                    32

1   an effective carrier. Native human proteins are preferred as TSTs, but recombinant peptides as well as organic or synthetic molecules resulting from drug screens may be equally effective in particular situations.

     TSTs may be delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol;

6   transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration will be determined by the attending physician and will vary according to each specific situation.

11   Such determinations are made by considering multiple variables such as the condition to be treated, the TST to be administered, and the pharmacokinetic profile of the particular TST. Additional factors which may be taken into account include disease state (eg. severity) of the patient, age, weight, gender, diet, time and frequency of administration,

16   drug combination, reaction sensitivities, and tolerance/response to therapy. Long acting TST formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular TST.

     Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to

21   a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature. See US Patent No. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art will employ different formulations for different TSTs. Administration to lung cells may necessitate delivery in a manner

26   different from that to kidney or other cells.

     It is contemplated that conditions associated with altered HJAK2 expression are treatable with TSTs. These conditions, which specifically include, but are not limited to, anemia, arteriosclerosis, asthma, bronchitis, emphysema, gingivitis, inflammatory bowel disease, insulin-

31   dependent diabetes mellitus, leukemia, multiple endocrine neoplasias, osteoarthritis, osteoporosis, pulmonary fibrosis, rheumatoid arthritis, septic shock syndromes, and systemic lupus erythematosus may be specifically diagnosed by the tests discussed above. In addition, such tests may be used to monitor treatment.

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1   All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with

6   specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the

11   following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Coleman, Roger
               Stuart, Susan G.

    (ii) TITLE OF INVENTION: A NOVEL HUMAN JAK2 KINASE HOMOLOG

   (iii) NUMBER OF SEQUENCES: 2

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    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

   (viii) ATTORNEY/AGENT INFORMATION:
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        (B) REGISTRATION NUMBER: 33954
        (C) REFERENCE/DOCKET NUMBER: PF-0049 US

    (ix) TELECOMMUNICATION INFORMATION:
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        (B) TELEFAX: 415-852-0195

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 4482 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
        (A) LIBRARY: Placenta
        (B) CLONE: 179527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCACGCGTCC GGTTGCCAAC CCGCAGGCGA CTGGGCGCTT CATCCCACCC TCACCCCTTT       60

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TGTGAGCGCG TTGTCCCCGG NCCCCGGGGC CACTTCCCCT CGGCCTAGNA GACTGGACTG      180

GGGAAGGACG GGTCTGTTGT ACCCGGGAGG TGGAAGGAAA AGCCGAAAGC GGAGAAGTGT      240

GCGGGAGGGG AGTCTCCGCG CGGAGGNAGA CCGGNCTCCT CCAGTGCAGG TTGTGCGCTG      300

GGGAGCCAGC CASGGCAAAT GTTCTGAAAA AGACTCTGCA TGGGAATGGC CTGCCTTACG      360

ATGACAGAAA TGGAGGGAAC ATCCACCTCT TCTATATATC AGAATGGTGA TATTTCTGGA      420

AATGCCAATT CTATGAAGCA AATAGATCCA GTTCTTCAGG TGTATCTTTA CCATTCCCTT      480

GGGAAATCTG AGGCAGATTA TCTGACCTTT CCATCTGGGG AGTATGTTGG AGAAGAAATC      540

TGTATTGCTG CTTCTAAAGC TTGTGGTATC ACACCTGTGT ATCATAATAT GTTTGCTTTA      600

ATGAGTGAAA CAGAAAGGAT CTGGTATCCA CCCAACCATG TCTTCCATAT AGATGAGTCA      660

ACCAGGCATA ATGTACTCTA CAGAATAAGA TTTTACTTTC CTCGTTGGTA TTGCAGTGGC      720

AGCAACAGAG CCTATCGGCA TGGAATATCT CGAGGTGCTG AAGCTCCTCT TCTTGATGAC      780

TTTGTCATGT CTTACCTCTT TGCTCAGTGG CGGCATGATT TTGTGCATGG ATGGATAAAA      840

GTACCTGTGA CTCATGAAAC ACAGGAAGAA TGTCTTGGGA TGACAGTGTT AGATATGATG      900

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CGAATAAGGT ACAGATTTCG CAGATTTATT CAGCAATTCA GCCAATGCAA AGCCACTGCC     1080

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GAGAAATTTG AAGTAAAAGA ACCTGGAAGT GGTCCTTCAG GTGAGGAGAT TTTTGCAACC     1200

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ACCCTTATTC ATGGGAATGT ATGTGCCAAA AATATTCTGC TTATCAGAGA AGAAGACAGG      2400

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AGTGGAGGAG ATAAACCTCT AAGTGCTCTG GATTCTCAAA GAAAGCTACA ATTTTATGAA      2640

GATAGGCATC AGCTTCCTGC ACCAAAGTGG GCAGAATTAG CAAACCTTAT AAATAATTGT      2700

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ACTGAAGAGC ACCTAAGAGA CTTTGAAAGG GAAATTGAAA TCCTGAAATC CCTACAGCAT    3060

GACAACATTG TAAAGTACAA GGGAGTGTGC TACAGTGCTG GTCGGCGTAA TCTAAAATTA    3120

ATTATGGAAT ATTTACCATA TGGAAGTTTA CGAGACTATC TTCAAAAACA TAAAGAACGG    3180

ATAGATCACA TAAAACTTCT GCAGTACACA TCTCAGATAT GCAAGGGTAT GGAGTATCTT    3240

GGTACAAAAA GGTATATCCA CAGGGATCTG GCAACGAGAA ATATATTGGT GGAGAACGAG    3300

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GACAAACAAG GACAGATGAT CGTGTTCCAT TTGATAGAAC TTTTGAAGAA TAATGGAAGA    3600

TTACCAAGAC CAGATGGATG CCCAGATGAG ATCTATATGA TCATGACAGA ATGCTGGAAC    3660

AATAATGTAA ATCAACGCCC CTCCTTTAGG GATCTAGCTC TTCGAGTGGA TCAAATAAGG    3720

GATAACATGG CTGGATGAAA GAAATGACCT TCATTCTGAG ACCAAAGTAG ATTTACAGAA    3780

CAAAGTTTTA TATTTCACAT TGCTGTGGAC TATTATTACA TATATCATTA TTATATAAAT    3840

CATGATGCTA GCCAGCAAAG ATGTGAAAAT ATCTGCTCAA AACTTTCAAA GTTTAGTAAG    3900

TTTTTCTTCA TGAGGCCACC AGTAAAAGAC ATTAATGAGA ATTCCTTAGC AAGGATTTTG    3960

TAAGAAGTTT CTTAAACATT GTCAGTTAAC ATCACTCTTG TCTGGCAAAA GAAAAAAAAT    4020

AGACTTTTTC AACTCAGCTT TTTGAGACCT GAAARAATTA TTATGTAAAT TTTGCAATGT    4080

TAAAGATGCA CAGAATATGT ATGTATAGTT TTTACCACAG TGGATGTATA ATACCTTGGC    4140

ATCTTGTGTG ATGTTTAACA CACATGAGGG CTGGTGTTCA TTAATACTGT TTTCTAATTT    4200

TTCCATGGTT AATCTATAAT TAATTACTTC ACTAAACAAA CAAATTAAGA TGTTCAGATA    4260
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ATTGAATAAG TACCTTTGTG TCCTTGTTCA TTTATATCGC TGGCCAGCAT TATAAGCAGG   4320

TGTATACTTT TAGCTTGTAG TTCCATGTAC TGTAAATATT TTTCACATAA AGGGAACAAA   4380

TGTCTAGTTT TATTTGTATA GGAAATTTGC CCTGACCCTA AATAATACAT TTTGAAATGA   4440

AACAAGCTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AG   4482

(2) INFORMATION FOR SEQ ID NO:2:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 1132 amino acids
         (B) TYPE: amino acid
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr
1   5   10   15

Ser Ser Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met
          20   25   30

Lys Gln Ile Asp Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly
        35   40   45

Lys Ser Glu Ala Asp Tyr Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly
      50   55   60

Glu Glu Ile Cys Ile Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65   70   75   80

Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
        85   90   95

Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asn Val
        100   105   110

Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser Gly Ser
        115   120   125

Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu
        130   135   140

Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp
145   150   155   160

39

Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
            165   170   175

Glu Cys Leu Gly Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu
            180   185   190

Asn Asp Gln Thr Pro Leu Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr
            195   200   205

Phe Leu Pro Gln Cys Ile Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
            210   215   220

Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225   230   235   240

Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu Lys Tyr Leu Ile
            245   250   255

Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe Glu Val
            260   265   270

Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
            275   280   285

Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys
            290   295   300

Glu Ser Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe
305   310   315   320

Pro Asn Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser
            325   330   335

Asn Glu Ser Arg Val Val Thr Ile His Lys Gln Asp Gly Lys Asn Leu
            340   345   350

Glu Ile Glu Leu Ser Ser Leu Arg Glu Ala Leu Ser Phe Val Ser Leu
            355   360   365

Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp Ala His His Tyr Leu Cys
            370   375   380

Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile Gln Ser Asn Cys
385   390   395   400

His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys Lys Ala
            405   410   415

Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
            420   425   430

Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu
    435   440   445

Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu
    450   455   460

Ser Gly Thr Lys Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys
465   470   475   480

Tyr Gln Met Glu Thr Val Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr
            485   490   495

Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
         500   505   510

Arg Thr Asn Gly Val Ser Asp Val Pro Thr Ser Pro Thr Leu Gln Arg
    515   520   525

Pro Thr His Met Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
    530   535   540

Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys Ile Phe
545   550   555   560

Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr
         565   570   575

Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu
         580   585   590

Ser Phe Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His
    595   600   605

Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu
    610   615   620

Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
625   630   635   640

Asn Lys Asn Cys Ile Asn Ile Leu Trp Lys Leu Glu Val Ala Lys Gln
         645   650   655

Leu Ala Trp Ala Met His Phe Leu Glu Glu Asn Thr Leu Ile His Gly
         660   665   670

Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Lys
         675   680   685

Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
    690   695   700

Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
705   710   715   720

Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
            725   730   735

Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
        740   745   750

Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
        755   760   765

Arg His Gln Leu Pro Ala Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile
    770   775   780

Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ser Phe Arg Ala
785   790   795   800

Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
            805   810   815

Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
            820   825   830

Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
        835   840   845

Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
        850   855   860

Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
865   870   875   880

Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
            885   890   895

Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
            900   905   910

Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile
        915   920   925

Met Glu Tyr Leu Pro Tyr Gly Ser Leu Arg Asp Tyr Leu Gln Lys His
        930   935   940

Lys Glu Arg Ile Asp His Ile Lys Leu Leu Gln Tyr Thr Ser Gln Ile
945   950   955   960

Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
            965   970   975

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Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
        980   985   990

Gly Ile Leu Gly * Pro Lys Ser Cys His Lys Thr Lys Asn Thr Ile
        995   1000   1005

Lys * Lys Asn Leu Val Lys Val Pro Tyr Ser Gly Met Leu Gln Asn
   1010   1015   1020

His * Gln Arg Ala Ser Phe Leu Trp Pro Gln Met Phe Gly Ala Leu
1025   1030   1035   1040

Glu Trp Phe Cys Met Asn Phe Ser His Thr Leu Arg Arg Val Lys Val
        1045   1050   1055

His Gln Arg Asn Leu Cys Val * Leu Ala Met Thr Asn Lys Asp Arg
        1060   1065   1070

  * Ser Cys Ser Ile *   * Asn Phe * Arg Ile Met Glu Asp Tyr
       1075   1080   1085

Gln Asp Gln Met Asp Ala Gln Met Arg Ser Ile * Ser * Gln Asn
    1090   1095   1100

Ala Gly Thr Ile Met * Ile Asn Ala Pro Pro Leu Gly Ile * Leu
1105   1110   1115   1120

Phe Glu Trp Ile Lys * Gly Ile Thr Trp Leu Asp
        1125   1130

1

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide of SEQ ID NO:2, or the complement of said polynucleotide.

2. The polynucleotide of Claim 1 comprising the nucleic acid sequence

6 for a novel human Jak2 kinase (hjak2) of SEQ ID NO:1.

3. An antisense molecule comprising the complement of the polynucleotide of Claim 2 or a portion thereof.

4. A pharmaceutical composition comprising the antisense molecule of Claim 3 and a pharmaceutically acceptable excipient.

11 5. A method of treating a subject with a condition associated with altered hjak2 expression comprising administering an effective amount of the pharmaceutical composition of Claim 4 to the subject.

6. A diagnostic composition comprising an oligomer of the polynucleotide of Claim 2.

16 7. A diagnostic test for a condition associated with altered hjak2 expression comprising the steps of:

a) providing a biological sample;

b) combining the biological sample and the diagnostic composition of Claim 6;

21 c) allowing hybridization to occur between the biological sample and the diagnostic composition under suitable conditions;

d) measuring the amount of hybridization to obtain a sample value; and

e) comparing the sample value with standard values to determine

26 whether hjak2 expression is altered.

8. An expression vector comprising the polynucleotide of Claim 1.

9. A host cell transformed with the expression vector of Claim 8.

10. A method for producing a polypeptide, said method comprising the steps of:

31 a) culturing the host cell of Claim 9 under conditions suitable for the expression of

the polypeptide; and

b) recovering the polypeptide from the host cell culture.

11. A purified polypeptide (HJAK2) comprising the amino acid sequence of

36 SEQ ID NO:2.

44

12.   A diagnostic composition comprising the polypeptide of Claim 11 or a portion thereof.

13.   A pharmaceutical composition comprising the polypeptide of Claim 11 and a pharmaceutically acceptable excipient.

14.   A method of treating a subject with a condition associated with altered HJAK2 expression comprising administering an effective amount of the pharmaceutical composition of Claim 13 to the subject.

15.   An antibody specific for the purified polypeptide of Claim 11, or portion thereof.

16.   A diagnostic composition comprising the antibody of Claim 15.

17.   A diagnostic test for a condition associated with altered HJAK2 expression comprising the steps of:

a)   providing a biological sample;

b)   combining the biological sample and the antibody of Claim 15 under conditions suitable for complex formation;

c)   measuring the amount of complex formation between HJAK2 and the antibody to obtain a sample amount; and

d)   comparing the amount of complex formation in the sample with standard amounts of complex formation, wherein a variation between the sample amount and standard amounts of complex formation establishes the presence of the condition.

18.   A method of screening a plurality of compounds for specific binding affinity with the polypeptide of Claim 11 or any portion thereof comprising the steps of:

a) providing a plurality of compounds;

b) combining HJAK2 with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and

c) detecting binding of HJAK2 to each of the plurality of compounds, thereby identifying the compounds which specifically bind HJAK2.

PF-0049P

ABSTRACT

A NOVEL HUMAN JAK2 KINASE

The present invention provides a polynucleotide (hjak2) which identifies and encodes a novel human Jak2 kinase (HJAK2) which was expressed in the placenta. The present invention also provides for antisense molecules and oligomers designed from the nucleotide sequence or its antisense. The invention further provides genetically engineered expression vectors and host cells for the production of purified HJAK2 peptide, antibodies capable of binding to HJAK2, inhibitors which bind to HJAK2 and pharmaceutical compositions based on HJAK2 specific antibodies or inhibitors. The invention specifically provides for diagnostic assays based on altered hjak2 expression and which allow identification of such a condition. These assays utilize probes which comprise oligomers, fragments, or portions of hjak2 or its regulatory elements or antibodies specifically binding HJAK2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: A NOVEL HUMAN JAK2 KINASE

Serial No.:   To Be Assigned   Filing Date: Herewith

Examiner: To Be Assigned   Group Art Unit: To Be Assigned

Official Draftsman
Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

SUBMISSION OF FORMAL DRAWINGS

Sir:

Transmitted herewith are Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 2A, 2B, 2C, 2D, 2E,

as thirteen (13) sheets of formal drawings for this application. Each sheet of drawing indicates

the identifying indicia suggested in 37 CFR Section 1.84(c) on the reverse side of the drawings.

Applicants believe that no fee is due with this paper. However, if the Commissioner

determines that a fee is necessary, the Commissioner is hereby authorized to charge any

additional fees associated with this communication or credit any overpayment to Incyte

Pharmaceuticals, Inc. Deposit Account No. **09-0108. A duplicate copy of this communication**

is enclosed.

50775   1

If there are any questions regarding the above, the Examiner is invited to call the undersigned at 650-855-0555.

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Date: *December 9, 1999* *Susan K. Sather*

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Nucleotide and deduced amino acid sequence:

```
              9            18           27           36           45           54
5' ATG GGA ATG  GCC TGC CTT  ACG ATG ACA  GAA ATG GAG  GGA ACA TCC  ACC TCT TCT
   Met Gly Met  Ala Cys Leu  Thr Met Thr  Glu Met Glu  Gly Thr Ser  Thr Ser Ser

             63           72           81           90           99          108
   ATA TAT CAG  AAT GGT GAT  ATT TCT GGA  AAT GCC AAT  TCT ATG AAG  CAA ATA GAT
   Ile Tyr Gln  Asn Gly Asp  Ile Ser Gly  Asn Ala Asn  Ser Met Lys  Gln Ile Asp

            117          126          135          144          153          162
   CCA GTT CTT  CAG GTG TAT  CTT TAC CAT  TCC CTT GGG  AAA TCT GAG  GCA GAT TAT
   Pro Val Leu  Gln Val Tyr  Leu Tyr His  Ser Leu Gly  Lys Ser Glu  Ala Asp Tyr

            171          180          189          198          207          216
   CTG ACC TTT  CCA TCT GGG  GAG TAT GTT  GGA GAA ATC  TGT ATT GCT  GCT TCT
   Leu Thr Phe  Pro Ser Gly  Glu Tyr Val  Gly Glu Ile  Cys Ile Ala  Ala Ser

            225          234          243          252          261          270
   AAA GCT TGT  GGT ATC ACA  CCT GTG TAT  CAT AAT ATG  TTT GCT TTA  ATG AGT GAA
   Lys Ala Cys  Gly Ile Thr  Pro Val Tyr  His Asn Met  Phe Ala Leu  Met Ser Glu

            279          288          297          306          315          324
   ACA GAA ATC  TGG TAT CCA  AAC CAT GTC  TTC CAT ATA  GAT GAG TCA  ACC
   Thr Glu Ile  Trp Tyr Pro  Asn His Val  Phe His Ile  Asp Glu Ser  Thr

            333          342          351          360          369          378
   AGG CAT AAT  GTA CTC TAC  AGA ATA CTC  TTT TAC CCT  CGT TGG TAT  TGC AGT
   Arg His Asn  Val Leu Tyr  Arg Ile Leu  Phe Tyr Pro  Arg Trp Tyr  Cys Ser

            387          396          405          414          423          432
   GGC AGC AGA  GCC TAT CGG  CAT GGA ATA  TCT CGA GGT  GCT GAA GCT  CCT CTT
   Gly Ser Arg  Ala Tyr Arg  His Gly Ile  Ser Arg Gly  Ala Glu Ala  Pro Leu

            441          450          459          468          477          486
   CTT GAT GAC  TTT GTC ATG  TCT TAC CTC  TTT GCT CAG  TGG CGG CAT  GAT TTT GTG
   Leu Asp Asp  Phe Val Met  Ser Tyr Leu  Phe Ala Gln  Trp Arg His  Asp Phe Val
```

FIGURE 1A

```
      495       504               513               522               531               540
CAT GGA   ATA AAA GTA CCT   GTG ACT CAT GAA   CAG GAA TGT CTT GGG
His Gly   Ile Lys Val Pro   Val Thr His Glu   Gln Glu Cys Leu Gly

      549       558               567               576               585               594
ATG ACA   GTG TTA GAT ATG   ATG AGA ATA GCC   AAA GAA AAC GAT ACC CCA CTG
Met Thr   Val Leu Asp Met   Met Arg Ile Ala   Lys Glu Asn Asp Thr Pro Leu

      603       612               621               630               639               648
GCC ATC   TAT AAC TCT ATC   AGC TAC TTA CCA   CAA TGT ATT CGA GCA
Ala Ile   Tyr Asn Ser Ile   Ser Tyr Leu Pro   Gln Cys Ile Arg Ala

      657       666               675               684               693               702
AAG ATC   CAA GAC TAT CAT   ATT TTG ACA AGG   AAG ATA AGG TAC AGA TTT CGC
Lys Ile   Gln Asp Tyr His   Ile Leu Thr Arg   Lys Ile Arg Tyr Arg Phe Arg

      711       720               729               738               747               756
AGA TTT   ATT CAG CAA TTC   AGC CAA TGC ACT   GCC AGA AAC TTG AAA CTT
Arg Phe   Ile Gln Gln Phe   Ser Gln Cys Ala   Thr Arg Asn Leu Lys Leu

      765       774               783               792               801               810
AAG TAT   CTT ATA AAT CTG   GAA ACT CTG CAG   TCT TTC TAC ACA GAG AAA TTT
Lys Tyr   Leu Ile Asn Leu   Glu Thr Leu Gln   Ser Phe Tyr Thr Glu Lys Phe

      819       828               837               846               855               864
GAA GTA   AAA GAA CCT GGA   AGT GGT CCT TCA   GGT GAG ATT TTT GCA ACC ATT
Glu Val   Lys Glu Pro Gly   Ser Gly Pro Ser   Gly Glu Ile Phe Ala Thr Ile

      873       882               891               900               909               918
ATA ATA   ACT GGA AAC GGT   GGA ATT CAG TGG   TCA AGA GGG AAA CAT AAA GAA AGT
Ile Ile   Thr Gly Asn Gly   Gly Ile Gln Trp   Ser Arg Gly Lys His Lys Glu Ser

      927       936               945               954               963               972
GAG ACA   CTG ACA GAA CAG   TTA TAT TGC GAT   TTT CCT AAT ATT ATT
Glu Thr   Leu Thr Glu Gln   Leu Tyr Cys Asp   Phe Pro Asn Ile Ile
```

FIGURE 1B

```
           981            990            999           1008           1017           1026
GAT GTC AGT ATT AAG GCA CAA AAC CAA GAG GGT TCA AAT GAA AGC CGA GTT GTA
Asp Val Ser Ile Lys Ala Gln Asn Gln Glu Gly Ser Asn Glu Ser Arg Val Val

          1035           1044           1053           1062           1071           1080
ACT ATC CAT AAG CAA GAT GGT AAA AAT CTG GAA ATT GAA CTT AGC TCA TTA AGG
Thr Ile His Lys Gln Asp Gly Lys Asn Leu Glu Ile Glu Leu Ser Ser Leu Arg

          1089           1098           1107           1116           1125           1134
GAA GCT TTG TCT TTC GTG TCA TTA ATT GAT GGA TAT TAT AGA TTA ACT GCA GAT
Glu Ala Leu Ser Phe Val Ser Leu Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp

          1143           1152           1161           1170           1179           1188
GCA CAT CAT TAC CTC TGT AAA GAA GTA GCA CCT CCA GCC GTG CTT GAA AAT ATA
Ala His His Tyr Leu Cys Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile

          1197           1206           1215           1224           1233           1242
CAA AGC AAC TGT CAT GGC CCA ATT TCG ATG GAT TTT GCC ATT AGT AAA CTG AAG
Gln Ser Asn Cys His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys

          1251           1260           1269           1278           1287           1296
AAA GCA GGT AAT CAG ACT GGA CTG TAT GTA CTT CGA TGC AGT CCT AAG GAC TTT
Lys Ala Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe

          1305           1314           1323           1332           1341           1350
AAT AAA TAT TTT TTG ACT TTT GCT GTC GAG CGA GAA AAT GTC ATT GAA TAT AAA
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu Tyr Lys

          1359           1368           1377           1386           1395           1404
CAC TGT TTG ATT ACA AAA AAT GAG AAT GAA GAG TAC AAC CTC AGT GGG ACA AAG
His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu Ser Gly Thr Lys
```

FIGURE 1C

1413
        1422   1431   1440   1449   1458
AAG AAC TTC AGC AGT CTT AAA GAT CTT TTG AAT TGT TAC CAG ATG GAA ACT GTT
Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys Tyr Gln Met Glu Thr Val

1467
        1476   1485   1494   1503   1512
CGC TCA GAC AAT ATA ATT TTC CAG TTT ACT AAA TGC TGT CCC CCA AAG CCA AAA
Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr Lys Cys Cys Pro Pro Lys Pro Lys

1521
        1530   1539   1548   1557   1566
GAT AAA TCA AAC CTT CTA GTC TTC AGA ACG AAT GGT GTT TCT GAT GTA CCA ACC
Asp Lys Ser Asn Leu Leu Val Phe Arg Thr Asn Gly Val Ser Asp Val Pro Thr

1575
        1584   1593   1602   1611   1620
TCA CCA TTA CAG AGG CCT ACT CAT ATG AAC CAA ATG GTG TTT CAC AAA ATC
Ser Pro Leu Gln Arg Pro Thr His Met Asn Gln Met Val Phe His Lys Ile

1629
        1638   1647   1656   1665   1674
AGA AAT GAA GAT TTG ATA TTT AAT AGC CTT GGC CAA GGC ACT TTT ACA AAG
Arg Asn Glu Asp Leu Ile Phe Asn Ser Leu Gly Gln Gly Thr Phe Thr Lys

1683
        1692   1701   1710   1719   1728
ATT TTT AAA GGC GTA CGA AGA GAA GTA GGA GAC TAC GGT CAA CTG CAT GAA ACA
Ile Phe Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr

1737
        1746   1755   1764   1773   1782
GAA GTT CTT TTA AAA GTT CTG GAT AAA GCA CAC AGG AAC TAT TCA GAG TCT TTC
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu Ser Phe

1791
        1800   1809   1818   1827   1836
TTT GAA GCA GCA AGT ATG ATG AGC AAG CTT TCT CAC AAG CAT TTG GTT TTA AAT
Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His Leu Val Leu Asn

1845
        1854   1863   1872   1881   1890
TAT GGA GTA TGT GTC TGT GGA GAC GAG AAT ATT CTG GTT CAG GAG TTT GTA AAA
Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe Val Lys

FIGURE 1D

```
                1899        1908        1917        1926        1935        1944
TTT GGA TCA CTA GAT ACA TAT CTG AAA AAG AAT AAA AAT TGT ATA AAT ATA TTA
Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys Asn Lys Asn Cys Ile Asn Ile Leu

                1953        1962        1971        1980        1989        1998
TGG AAA CTT GAA GTT GCT CAG TTG GCA TGG GCC ATG CAT TTT CTA GAA GAA
Trp Lys Leu Glu Val Ala Gln Leu Ala Trp Ala Met His Phe Leu Glu Glu

                2007        2016        2025        2034        2043        2052
AAC ACC ATT CAT GGG AAT GTA TGT GCC AAA AAT ATT CTG CTT ATC AGA GAA
Asn Thr Ile His Gly Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu

                2061        2070        2079        2088        2097        2106
GAA GAC AAG ACA GGA AAT CCT TTC ATC AAA CTT CTT AGT GAT CCT GGC ATT
Glu Asp Lys Thr Gly Asn Pro Phe Ile Lys Leu Leu Ser Asp Pro Gly Ile

                2115        2124        2133        2142        2151        2160
AGT ATT ACA GTT TTG CCA AAG GAC ATT CTT CAG AGA ATA CCA TGG GTA CCA
Ser Ile Thr Val Leu Pro Lys Asp Ile Leu Gln Arg Ile Pro Trp Val Pro

                2169        2178        2187        2196        2205        2214
CCT GAA TGC ATT GAA AAT CCT AAA AAT TTA AAT TTG GCA ACA GAC AAA TGG AGT
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys Trp Ser

                2223        2232        2241        2250        2259        2268
TTT GGT ACC TTG TGG GAA ATC TGC AGT GGA GGA GAT AAA CCT CTA AGT GCT
Phe Gly Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys Pro Leu Ser Ala

                2277        2286        2295        2304        2313        2322
GAT TCT CAA AGA AAG CTA CAA TTT TAT GAA GAT AGG CAT CAG CTT CCT GCA
Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp Arg His Gln Leu Pro Ala

                2331        2340        2349        2358        2367        2376
CCA AAG TGG GCA TTA GCA AAC CTT ATA AAT AAT AAT TGT ATG GAT TAT GAA CCA
Pro Lys Trp Ala Leu Ala Asn Leu Ile Asn Asn Cys Met Asp Tyr Glu Pro
```

FIGURE 1E

2385　　　　2394　　　　2403　　　　2412　　　　2421　　　　2430
GAT TTC AGG CCT TCT AGA GCC ATC ATA CGA GAT CTT AAC AGT TTG TTT ACT
Asp Phe Arg Pro Ser Phe Arg Ala Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr

2439　　　　2448　　　　2457　　　　2466　　　　2475　　　　2484
CCA GAT TAT GAA CTA TTA ACA GAA AAT GAC ATG TTA CCA AAT ATG AGG ATA GGT
Pro Asp Tyr Glu Leu Leu Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly

2493　　　　2502　　　　2511　　　　2520　　　　2529　　　　2538
GCC TTG GGG TTT TCT GGT GCC TTT GAA GAC CGG GAT CCT ACA CAG TTT GAA GAG
Ala Leu Gly Phe Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu

2547　　　　2556　　　　2565　　　　2574　　　　2583　　　　2592
AGA CAT TTG AAA TTT CTA CAG CAA CTT GGC AAG GGT AAT TTT GGG AGT GTG GAG
Arg His Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu

2601　　　　2610　　　　2619　　　　2628　　　　2637　　　　2646
ATG TGC CGG TAT GAC CCT CTA CAG GAC AAC ACT GGG GAG GTC GTC GCT GTA AAA
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala Val Lys

2655　　　　2664　　　　2673　　　　2682　　　　2691　　　　2700
AAG CTT CAG CAT AGT ACT GAA GAG CAC CTA CAG AGA GAC TTT GAA AGG GAA ATT GAA
Lys Leu Gln His Ser Thr Glu Glu His Leu Gln Arg Asp Phe Glu Arg Glu Ile Glu

2709　　　　2718　　　　2727　　　　2736　　　　2745　　　　2754
ATC CTG AAA TCC CTA CAG CAT GAC AAC ATT GTA AAG TAC AAG GGA GTG TGC TAC
Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys Tyr Lys Gly Val Cys Tyr

2763　　　　2772　　　　2781　　　　2790　　　　2799　　　　2808
AGT GCT GGT CGG CGT AAT CTA AAA TTA ATT ATG GAA TAT TTA CCA TAT GGA AGT
Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile Met Glu Tyr Leu Pro Tyr Gly Ser

2817　　　　2826　　　　2835　　　　2844　　　　2853　　　　2862
TTA CGA GAC TAT CTT CAA AAA CAT AAA GAA CGG ATA GAT CAC ATA AAA CTT CTG
Leu Arg Asp Tyr Leu Gln Lys His Lys Glu Arg Ile Asp His Ile Lys Leu Leu

FIGURE 1F

```
      2871          2880          2889          2898          2907          2916
CAG TAC ACA   TCT CAG ATA   TGC AAG GGT   ATG GAG TAT   CTT GGT ACA   AAA AGG TAT
Gln Tyr Thr   Ser Gln Ile   Cys Lys Gly   Met Glu Tyr   Leu Gly Thr   Lys Arg Tyr

      2925          2934          2943          2952          2961          2970
ATC CAC AGG   GAT CTG GCA   ACG AGA AAT   ATA TTG GTG   GAG AAC AGA GTT
Ile His Arg   Asp Leu Ala   Thr Arg Asn   Ile Leu Val   Glu Asn Arg Val

      2979          2988          2997          3006          3015          3024
AAA ATT GGR   GAT TTT GGG   TTA ACC AAA   GTC TTG CCA   CAA GAC AAA   GAA TAC TAT
Lys Ile Gly   Asp Phe Gly   Leu Thr Lys   Val Leu Pro   Gln Asp Lys   Glu Tyr Tyr

      3033          3042          3051          3060          3069          3078
AAA GTA AAA   CCT GGT GAA   AGT CCC ATA   TTC TGG TAT   GCT CCA GAA   TCA CTG
Lys Val Lys   Pro Gly Glu   Ser Pro Ile   Phe Trp Tyr   Ala Pro Glu   Ser Leu

      3087          3096          3105          3114          3123          3132
ACA GAG AGC   AAG TTT TCT   GTG GCC TCA   GAT GTT TGG   AGC TTT GGA   GTG GTT CTG
Thr Glu Ser   Lys Phe Ser   Val Ala Ser   Asp Val Trp   Ser Phe Gly   Val Val Leu

      3141          3150          3159          3168          3177          3186
TAT GAA CTT   TTC ACA TAC   ATT GAG AAG   AGT AAA AGT   CCA CCA GCG   GAA TTT ATG
Tyr Glu Leu   Phe Thr Tyr   Ile Glu Lys   Ser Lys Ser   Pro Pro Ala   Glu Phe Met

      3195          3204          3213          3222          3231          3240
CGT ATT GGC   AAT GAC AAA   CAA GGA CAG   ATG ATC GTG   TTC CAT TTG   ATA GAA
Arg Ile Gly   Asn Asp Lys   Gln Gly Gln   Met Ile Val   Phe His Leu   Ile Glu

      3249          3258          3267          3276          3285          3294
CTT TTG AAG   AAT AAT GGA   AGA TTA CCA   AGA CCA GGA   TGC CCA GAT   GAG ATC
Leu Leu Lys   Asn Asn Gly   Arg Leu Pro   Arg Pro Gly   Cys Pro Asp   Glu Ile

      3303          3312          3321          3330          3339          3348
TAT ATC ATG   ACA GAA TGC   TGG AAC AAT   AAT GTA AAT   CAA AAT CAA   CGC CCC TCC TTT
Tyr Met Ile   Thr Glu Cys   Trp Asn Asn   Asn Val Asn   Gln Asn Gln   Arg Pro Ser Phe
```

FIGURE 1G

```
       3357     3366     3375     3384     3393
AGG GAT CTA GCT CTT CGA GTG GAT CAA ATA AGG GAT AAC ATG GCT GGA TGA ***  3'
Arg Asp Leu Ala Leu Arg Val Asp Gln Ile Arg Asp Asn Met Ala Gly ***
```

FIGURE 1H

```
  1   M G M A C L T M T E M E G T S S S I Y Q N G D I S G N A N   179527
  1   M G M A C L T M T E M E A T S T S P V H Q N G D I P G S A N   GI 409584

 31   S M K Q I D P V L Q V Y L Y H S L G K S E A D Y L T F P S G   179527
 31   S V K Q I E P V L Q V Y L Y H S L G Q A E G E Y L K F P S G   GI 409584

 61   E Y V G E E I C I A A S K A C G I T P V Y H N M F A L M S E   179527
 61   E Y V A E E I C V A A S K A C G I T P V Y H N M F A L M S E   GI 409584

 91   T E R I W Y P P N H V F H I D E S T R H N V L Y R I R F Y F   179527
 91   T E R I W Y P P N H V F H I D E S T R H D I L Y R I R F Y F   GI 409584

121   P R W Y C S G S N R A Y R H G I S R G A E A P L L D D F V M   179527
121   P H W Y C S G S S R T Y R Y G V S R G A E A P L L D D F V M   GI 409584

151   S Y L F A Q W R H D F V H G W I K V P V T H E T Q E E C L G   179527
151   S Y L F V Q W R H D F V H G W I K V P V T H E T Q E E C L G   GI 409584

181   M T V L D M M R I A K E N D Q T P L A I Y N S I S Y K T F L   179527
181   M A V L D M M R I A K E K D Q T P L A V Y N S V S Y K T F L   GI 409584

211   P Q C I R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F   179527
211   P K C V R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F   GI 409584
```

FIGURE 2A

```
241  S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E K F   179527
241  S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F   GI 409584

271  E V K E P G S G P S G E E I F A T I I I T G N G G I Q W S R   179527
271  E V K E S A R G P S G E E I F A T I I I T G N G G I Q W S R   GI 409584

301  G K H K E S E T L T E Q D L Q L Y C D F P N I I D V S I K Q   179527
301  G K H K E S E T L T E Q D V Q L Y C D F P D I D V S I K Q   GI 409584

331  A N Q E G S N E S R V V T I H K Q D G K N L E I E L S S L R   179527
331  A N Q E C S N E S R I V T V H K Q D G K V L E I E L S S L K   GI 409584

361  E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P   179527
361  E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P   GI 409584

391  A V L E N I Q S N C H G P I S M D F A I S K L K K A G N Q T   179527
391  A V L E N I H S N C H G P I S M D F A I S K L K K A G N Q T   GI 409584

421  G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K   179527
421  G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K   GI 409584

451  H C L I T K N E N E E Y N L S G T K K N F S S L K D L L N C   179527
451  H C L I T K N E N G E Y N L S G T N R N F S N L K D L L N C   GI 409584
```

FIGURE 2B

```
481  Y Q M E T V R S D N I I F Q F T K C C P P K P K D K S N L L    179527
481  Y Q M E T V R S D S I I F Q F T K C C P P K P K D K S N L L    GI 409584

511  V F R T N G V S D V P T S P T L Q R P T H M N Q M V F H K I    179527
511  V F R T N G I S D V Q I S P T L Q R H N N V N Q M V F H K I    GI 409584

541  R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y    179527
541  R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y    GI 409584

571  G Q L H E T E V L L K V L D K A H R N Y S E S F F E A A S M    179527
571  G Q L H K T E V L L K V L D K A H R N Y S E S F F E A A S M    GI 409584

601  M S K L S H K H L V L N Y G V C V C G D E N I L V Q E F V K    179527
601  M S Q L S H K H L V L N Y G V C V C G E E N I L V Q E F V K    GI 409584

631  F G S L D T Y L K K N K N C I N I L W K L E V A K Q L A W A    179527
631  F G S L D T Y L K K N K N S I N I L W K L G V A K Q L A W A    GI 409584

661  M H F L E E N T L I H G N V C A K N I L L I R E E D R K T G    179527
661  M H F L E E K S L I H G N V C A K N I L L I R E E D R R T G    GI 409584

691  N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P    179527
691  N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P    GI 409584
```

FIGURE 2C

```
721  P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G   179527
721  P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G   GI 409584

751  D K P L S A L D S Q R K L Q F Y E D R H Q L P A P K W A E L   179527
751  D K P L S A L D S Q R K L Q F Y E D K H Q L P A P K W T E L   GI 409584

781  A N L I N N C M D Y E P D F R P S F R A I I R D L N S L F T   179527
781  A N L I N N C M D Y E P D F R P A F R A V I R D L N S L F T   GI 409584

811  P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D   179527
811  P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D   GI 409584

841  P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P   179527
841  P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P   GI 409584

871  L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E   179527
871  L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E   GI 409584

901  I L K S L Q H D N I V K Y K G V C Y S A G R R N L K L I M E   179527
901  I L K S L Q H D N I V K Y K G V C Y S A G R R N L R L I M E   GI 409584

931  Y L P Y G S L R D Y L Q K H K E R I D H I K L L Q Y T S Q I   179527
931  Y L P Y G S L R D Y L Q K H K E R I D H K K L L Q Y T S Q I   GI 409584
```

FIGURE 2D

961 C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V 179527
961 C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V GI 409584

991 K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W 179527
991 K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W GI 409584

1021 Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y 179527
1021 Y A P Q S L T E S K F S V A S D V W S F G V V L Y E L F T Y GI 409584

1051 I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E 179527
1051 I E K S K S P P V E F M R M I G N D K Q G Q M I V F H L I E GI 409584

1081 L L K N N G R L P R P D G C P D E I Y M I M T E C W N N N V 179527
1081 L L K S N G R L P R P E G C P D E I Y V I M T E C W N N N V GI 409584

1111 N Q R P S F R D L A L R V D Q I R D N M A G 179527
1111 S Q R P S F R D L S F - - G W I K C G - T V GI 409584

FIGURE 2E

```
              348            357            366            375            334            393
5' ATG GGA ATG GCC TGC CTT ACG ATG ACA GAA ATG GAG GGA ACA TCC ACC TCT TCT
   Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr Ser Ser

       402            411            420            429            438            447
   ATA TAT CAG AAT GGT GAT ATT TCT GGA AAT GCC AAT TCT ATG AAG CAA ATA GAT
   Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met Lys Gln Ile Asp

       456            465            474            483            492            501
   CCA GTT CTT CAG GTG TAT CTT TAC CAT TCC CTT GGG AAA TCT GAG GCA GAT TAT
   Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly Lys Ser Glu Ala Asp Tyr

       510            519            528            537            546            555
   CTG ACC TTT CCA TCT GGG GAG TAT GTT GGA GAA GAA ATC TGT ATT GCT GCT TCT
   Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly Glu Glu Ile Cys Ile Ala Ala Ser

       564            573            582            591            600            609
   AAA GCT TGT GGT ATC ACA CCT GTG TAT CAT AAT ATG TTT GCT TTA ATG AGT GAA
   Lys Ala Cys Gly Ile Thr Pro Val Tyr His Asn Met Phe Ala Leu Met Ser Glu

       618            627            636            645            654            663
   ACA GAA AGG ATC TGG TAT CCA CCC AAC CAT GTC TTC CAT ATA GAT GAG TCA ACC
   Thr Glu Arg Ile Trp Tyr Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr

       672            681            690            699            708            717
   AGG CAT AAT GTA CTC TAC AGA ATA AGA TTT TAC TTT CCT CGT TGG TAT TGC AGT
   Arg His Asn Val Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser

       726            735            744            753            762            771
   GGC AGC AAC AGA GCC TAT CGG CAT GGA ATA TCT CGA GGT GCT GAA GCT CCT CTT
   Gly Ser Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu

       780            789            798            807            816            825
   CTT GAT GAC TTT GTC ATG TCT TAC CTC TTT GCT CAG TGG CGG CAT GAT TTT GTG
   Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp Phe Val

       834            843            852            861            870            879
   CAT GGA TGG ATA AAA GTA CCT GTG ACT CAT GAA ACA CAG GAA GAA TGT CTT GGG
   His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu Glu Cys Leu Gly

       888            897            906            915            924            933
   ATG ACA GTG TTA GAT ATG ATG AGA ATA GCC AAA GAA AAC GAT CAA ACC CCA CTG
   Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu Asn Asp Gln Thr Pro Leu

       942            951            960            969            978            987
   GCC ATC TAT AAC TCT ATC AGC TAC AAG ACA TTC TTA CCA CAA TGT ATT CGA GCA
   Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr Phe Leu Pro Gln Cys Ile Arg Ala
```

FIGURE 1A

```
        996          1005          1014          1023          1032          1041
AAG ATC CAA GAC TAT CAT ATT TTG ACA AGG AAG CGA ATA AGG TAC AGA TTT CGC
Lys Ile Gln Asp Tyr His Ile Leu Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg


       1050          1059          1068          1077          1086          1095
AGA TTT ATT CAG CAA TTC AGC CAA TGC AAA GCC ACT GCC AGA AAC TTG AAA CTT
Arg Phe Ile Gln Gln Phe Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu


       1104          1113          1122          1131          1140          1149
AAG TAT CTT ATA AAT CTG GAA ACT CTG CAG TCT GCC TTC TAC ACA GAG AAA TTT
Lys Tyr Leu Ile Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe


       1158          1167          1176          1185          1194          1203
GAA GTA AAA GAA CCT GGA AGT GGT CCT TCA GGT GAG GAG ATT TTT GCA ACC ATT
Glu Val Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile


       1212          1221          1230          1239          1248          1257
ATA ATA ACT GGA AAC GGT GGA ATT CAG TGG TCA AGA GGG AAA CAT AAA GAA AGT
Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys Glu Ser


       1266          1275          1284          1293          1302          1311
GAG ACA CTG ACA GAA CAG GAT TTA CAG TTA TAT TGC GAT TTT CCT AAT ATT ATT
Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe Pro Asn Ile Ile


       1320          1329          1338          1347          1356          1365
GAT GTC AGT ATT AAG CAA GCA AAC CAA GAG GGT TCA AAT GAA AGC CGA GTT GTA
Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser Asn Glu Ser Arg Val Val


       1374          1383          1392          1401          1410          1419
ACT ATC CAT AAG CAA GAT GGT AAA AAT CTG GAA ATT GAA CTT AGC TCA TTA AGG
Thr Ile His Lys Gln Asp Gly Lys Asn Leu Glu Ile Glu Leu Ser Ser Leu Arg


       1428          1437          1446          1455          1464          1473
GAA GCT TTG TCT TTC GTG TCA TTA ATT GAT GGA TAT TAT AGA TTA ACT GCA GAT
Glu Ala Leu Ser Phe Val Ser Leu Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp


       1482          1491          1500          1509          1518          1527
GCA CAT CAT TAC CTC TGT AAA GAA GTA GCA CCT CCA GCC GTG CTT GAA AAT ATA
Ala His His Tyr Leu Cys Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile


       1536          1545          1554          1563          1572          1581
CAA AGC AAC TGT CAT GGC CCA ATT TCG ATG GAT TTT GCC ATT AGT AAA CTG AAG
Gln Ser Asn Cys His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys


       1590          1599          1608          1617          1626          1635
AAA GCA GGT AAT CAG ACT GGA CTG TAT GTA CTT CGA TGC AGT CCT AAG GAC TTT
Lys Ala Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
```

FIGURE 1B

```
      1644          1653          1662          1671          1680          1689
AAT AAA TAT TTT TTG ACT TTT GCT GTC GAG CGA GAA AAT GTC ATT GAA TAT AAA
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu Tyr Lys

      1698          1707          1716          1725          1734          1743
CAC TGT TTG ATT ACA AAA AAT GAG AAT GAA GAG TAC AAC CTC AGT GGG ACA AAG
His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu Ser Gly Thr Lys

      1752          1761          1770          1779          1788          1797
AAG AAC TTC AGC AGT CTT AAA GAT CTT TTG AAT TGT TAC CAG ATG GAA ACT GTT
Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys Tyr Gln Met Glu Thr Val

      1806          1815          1824          1833          1842          1851
CGC TCA GAC AAT ATA ATT TTC CAG TTT ACT AAA TGC TGT CCC CCA AAG CCA AAA
Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr Lys Cys Cys Pro Pro Lys Pro Lys

      1860          1869          1878          1887          1896          1905
GAT AAA TCA AAC CTT CTA GTC TTC AGA ACG AAT GGT GTT TCT GAT GTA CCA ACC
Asp Lys Ser Asn Leu Leu Val Phe Arg Thr Asn Gly Val Ser Asp Val Pro Thr

      1914          1923          1932          1941          1950          1959
TCA CCA ACA TTA CAG AGG CCT ACT CAT ATG AAC CAA ATG GTG TTT CAC AAA ATC
Ser Pro Thr Leu Gln Arg Pro Thr His Met Asn Gln Met Val Phe His Lys Ile

      1968          1977          1986          1995          2004          2013
AGA AAT GAA GAT TTG ATA TTT AAT GAA AGC CTT GGC CAA GGC ACT TTT ACA AAG
Arg Asn Glu Asp Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys

      2022          2031          2040          2049          2058          2067
ATT TTT AAA GGC GTA CGA AGA GAA GTA GGA GAC TAC GGT CAA CTG CAT GAA ACA

Ile Phe Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr

      2076          2085          2094          2103          2112          2121
GAA GTT CTT TTA AAA GTT CTG GAT AAA GCA CAC AGG AAC TAT TCA GAG TCT TTC
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu Ser Phe

      2130          2139          2148          2157          2166          2175
TTT GAA GCA GCA AGT ATG ATG AGC AAG CTT TCT CAC AAG CAT TTG GTT TTA AAT
Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His Leu Val Leu Asn

      2184          2193          2202          2211          2220          2229
TAT GGA GTA TGT GTC TGT GGA GAC GAG AAT ATT CTG GTT CAG GAG TTT GTA AAA
Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe Val Lys

      2238          2247          2256          2265          2274          2283
TTT GGA TCA CTA GAT ACA TAT CTG AAA AAG AAT AAA AAT TGT ATA AAT ATA TTA
Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys Asn Lys Asn Cys Ile Asn Ile Leu
```

FIGURE 1C

```
     2292          2301          2310          2319          2328          2337
TGG AAA CTT GAA GTT GCT AAA CAG TTG GCA TGG GCC ATG CAT TTT CTA GAA GAA
Trp Lys Leu Glu Val Ala Lys Gln Leu Ala Trp Ala Met His Phe Leu Glu Glu

     2346          2355          2364          2373          2382          2391
AAC ACC CTT ATT CAT GGG AAT GTA TGT GCC AAA AAT ATT CTG CTT ATC AGA GAA
Asn Thr Leu Ile His Gly Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu

     2400          2409          2418          2427          2436          2445
GAA GAC AGG AAG ACA GGA AAT CCT CCT TTC ATC AAA CTT AGT GAT CCT GGC ATT
Glu Asp Arg Lys Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile

     2454          2463          2472          2481          2490          2499
AGT ATT ACA GTT TTG CCA AAG GAC ATT CTT CAG GAG AGA ATA CCA TGG GTA CCA
Ser Ile Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro

     2508          2517          2526          2535          2544          2553
CCT GAA TGC ATT GAA AAT CCT AAA AAT TTA AAT TTG GCA ACA GAC AAA TGG AGT
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys Trp Ser

     2562          2571          2580          2589          2598          2607
TTT GGT ACC ACT TTG TGG GAA ATC TGC AGT GGA GGA GAT AAA CCT CTA AGT GCT
Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys Pro Leu Ser Ala

     2616          2625          2634          2643          2652          2661
CTG GAT TCT CAA AGA AAG CTA CAA TTT TAT GAA GAT AGG CAT CAG CTT CCT GCA
Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp Arg His Gln Leu Pro Ala

     2670          2679          2688          2697          2706          2715
CCA AAG TGG GCA GAA TTA GCA AAC CTT ATA AAT AAT TGT ATG GAT TAT GAA CCA
Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile Asn Asn Cys Met Asp Tyr Glu Pro

     2724          2733          2742          2751          2760          2769
GAT TTC AGG CCT TCT TTC AGA GCC ATC ATA CGA GAT CTT AAC AGT TTG TTT ACT
Asp Phe Arg Pro Ser Phe Arg Ala Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr

     2778          2787          2796          2805          2814          2823
CCA GAT TAT GAA CTA TTA ACA GAA AAT GAC ATG TTA CCA AAT ATG AGG ATA GGT
Pro Asp Tyr Glu Leu Leu Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly

     2832          2841          2850          2859          2868          2877
GCC TTG GGG TTT TCT GGT GCC TTT GAA GAC CGG GAT CCT ACA CAG TTT GAA GAG
Ala Leu Gly Phe Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu

     2886          2895          2904          2913          2922          2931
```

FIGURE 1D

```
AGA CAT TTG AAA TTT CTA CAG CAA CTT GGC AAG GGT AAT TTT GGG AGT GTG GAG
Arg His Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu

        2940      2949      2958      2967      2976      2985
ATG TGC CGG TAT GAC CCT CTA CAG GAC AAC ACT GGG GAG GTG GTC GCT GTA AAA
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala Val Lys

        2994      3003      3012      3021      3030      3039
AAG CTT CAG CAT AGT ACT GAA GAG CAC CTA AGA GAC TTT GAA AGG GAA ATT GAA
Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu Arg Glu Ile Glu

        3048      3057      3066      3075      3084      3093
ATC CTG AAA TCC CTA CAG CAT GAC AAC ATT GTA AAG TAC AAG GGA GTG TGC TAC
Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys Tyr Lys Gly Val Cys Tyr

        3102      3111      3120      3129      3138      3147
AGT GCT GGT CGG CGT AAT CTA AAA TTA ATT ATG GAA TAT TTA CCA TAT GGA AGT
Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile Met Glu Tyr Leu Pro Tyr Gly Ser

        3156      3165      3174      3183      3192      3201
TTA CGA GAC TAT CTT CAA AAA CAT AAA GAA CGG ATA GAT CAC ATA AAA CTT CTG
Leu Arg Asp Tyr Leu Gln Lys His Lys Glu Arg Ile Asp His Ile Lys Leu Leu

        3210      3219      3228      3237      3246      3255
CAG TAC ACA TCT CAG ATA TGC AAG GGT ATG GAG TAT CTT GGT ACA AAA AGG TAT
Gln Tyr Thr Ser Gln Ile Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr

        3264      3273      3282      3291      3300      3309
ATC CAC AGG GAT CTG GCA ACG AGA AAT ATA TTG GTG GAG AAC GAG AAC AGA GTT
Ile His Arg Asp Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val

        3318      3327      3336      3345      3354      3363
AAA ATT GGR GAT TTT GGG TTA ACC AAA GTC TTG CCA CAA GAC AAA GAA TAC TAT
Lys Ile Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr

        3372      3381      3390      3399      3408      3417
AAA GTA AAA GAA CCT GGT GAA AGT CCC ATA TTC TGG TAT GCT CCA GAA TCA CTG
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Glu Ser Leu

        3426      3435      3444      3453      3462      3471
ACA GAG AGC AAG TTT TCT GTG GCC TCA GAT GTT TGG AGC TTT GGA GTG GTT CTG
Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe Gly Val Val Leu

        3480      3489      3498      3507      3516      3525
TAT GAA CTT TTC ACA TAC ATT GAG AAG AGT AAA AGT CCA CCA GCG GAA TTT ATG
Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser Pro Pro Ala Glu Phe Met

        3534      3543      3552      3561      3570      3579
CGT ATG ATT GGC AAT GAC AAA CAA GGA CAG ATG ATC GTG TTC CAT TTG ATA GAA
Arg Met Ile Gly Asn Asp Lys Gln Gly Gln Met Ile Val Phe His Leu Ile Glu
```

FIGURE 1E

```
       3588          3597          3606          3615          3624          3633
CTT TTG AAG AAT AAT GGA AGA TTA CCA AGA CCA GAT GGA TGC CCA GAT GAG ATC
Leu Leu Lys Asn Asn Gly Arg Leu Pro Arg Pro Asp Gly Cys Pro Asp Glu Ile

       3642          3651          3660          3669          3678          3687
TAT ATG ATC ATG ACA GAA TGC TGG AAC AAT AAT GTA AAT CAA CGC CCC TCC TTT
Tyr Met Ile Met Thr Glu Cys Trp Asn Asn Asn Val Asn Gln Arg Pro Ser Phe

       3696          3705          3714          3723          3732
AGG GAT CTA GCT CTT CGA GTG GAT CAA ATA AGG GAT AAC ATG GCT GGA TGA   3'
Arg Asp Leu Ala Leu Arg Val Asp Gln Ile Arg Asp Asn Met Ala Gly ***
```

FIGURE 1F

```
MGMACLTMTEME.TSTS...QNGDI.G.AN
MGMACLTMTEMEGTSTSSVHQNGDISGSAN
         10        20        30

 1  MGMACLTMTEMEATSTSPVHQNGDIPGSAN
 1  MGMACLTMTEMEGTSTSSIYQNGDISGNAN

    S.KQI.PVLQVYLYHSLG..E..YL.FPSG
    SVKQIDPVLQVYLYHSLGQAEGDYLTFPSG
             40        50        60

31  SVKQIEPVLQVYLYHSLGQAEGEYLKFPSG
31  SMKQIDPVLQVYLYHSLGKSEADYLTFPSG

    EYV.EEIC.AASKACGITPVYHNMFALMSE
    EYVGEEICVAASKACGITPVYHNMFALMSE
             70        80        90

61  EYVAEEICVAASKACGITPVYHNMFALMSE
61  EYVGEEICIAASKACGITPVYHNMFALMSE

    TERIWYPPNHVFHIDESTRH..LYRIRFYF
    TERIWYPPNHVFHIDESTRHDVLYRIRFYF
            100       110       120

91  TERIWYPPNHVFHIDESTRHDILYRIRFYF
91  TERIWYPPNHVFHIDESTRHNVLYRIRFYF

    P.WYCSGS.R.YR.G.SRGAEAPLLDDFVM
    PHWYCSGSSRAYRHGVSRGAEAPLLDDFVM
            130       140       150

121  PHWYCSGSSRTYRYGVSRGAEAPLLDDFVM
121  PRWYCSGSNRAYRHGISRGAEAPLLDDFVM

    SYLF.QWRHDFVHGWIKVPVTHETQEECLG
    SYLFAQWRHDFVHGWIKVPVTHETQEECLG
            160       170       180

151  SYLFVQWRHDFVHGWIKVPVTHETQEECLG
151  SYLFAQWRHDFVHGWIKVPVTHETQEECLG

    M.VLDMMRIAKE.DQTPLA.YNS.SYKTFL
    MAVLDMMRIAKENDQTPLAVYNSVSYKTFL
            190       200       210

181  MAVLDMMRIAKEKDQTPLAVYNSVSYKTFL
181  MTVLDMMRIAKENDQTPLAIYNSISYKTFL
```

FIGURE 2A

```
          P . C . R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
          P Q C V R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
                    |                   |                   |
                   220                 230                 240
    211   P K C V R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F
    211   P Q C I R A K I Q D Y H I L T R K R I R Y R F R R F I Q Q F

          S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E . F
          S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F
                    |                   |                   |
                   250                 260                 270
    241   S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F
    241   S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E K F

          E V K E . . . G P S G E E I F A T I I I T G N G G I Q W S R
          E V K E S G S G P S G E E I F A T I I I T G N G G I Q W S R
                    |                   |                   |
                   280                 290                 300
    271   E V K E S A R G P S G E E I F A T I I I T G N G G I Q W S R
    271   E V K E P G S G P S G E E I F A T I I I T G N G G I Q W S R

          G K H K E S E T L T E Q D . Q L Y C D F P . I I D V S I K Q
          G K H K E S E T L T E Q D V Q L Y C D F P D I I D V S I K Q
                    |                   |                   |
                   310                 320                 330
    301   G K H K E S E T L T E Q D V Q L Y C D F P D I I D V S I K Q
    301   G K H K E S E T L T E Q D L Q L Y C D F P N I I D V S I K Q

          A N Q E . S N E S R . V T . H K Q D G K . L E I E L S S L .
          A N Q E G S N E S R V V T V H K Q D G K V L E I E L S S L K
                    |                   |                   |
                   340                 350                 360
    331   A N Q E G S N E S R I V T V H K Q D G K V L E I E L S S L K
    331   A N Q E G S N E S R V V T I H K Q D G K N L E I E L S S L R

          E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
          E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
                    |                   |                   |
                   370                 380                 390
    361   E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
    361   E A L S F V S L I D G Y Y R L T A D A H H Y L C K E V A P P
```

FIGURE 2B

```
A V L E N I . S N C H G P I S M D F A I S K L K K A G N Q T
A V L E N I Q S N C H G P I S M D F A I S K L K K A G N Q T
                    400                 410                 420
391  A V L E N I H S N C H G P I S M D F A I S K L K K A G N Q T
391  A V L E N I Q S N C H G P I S M D F A I S K L K K A G N Q T

G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
                    430                 440                 450
421  G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K
421  G L Y V L R C S P K D F N K Y F L T F A V E R E N V I E Y K

H C L I T K N E N . E Y N L S G T . . N F S . L K D L L N C
H C L I T K N E N G E Y N L S G T N K N F S S L K D L L N C
                    460                 470                 480
451  H C L I T K N E N G E Y N L S G T N R N F S N L K D L L N C
451  H C L I T K N E N E E Y N L S G T K K N F S S L K D L L N C

Y Q M E T V R S D . I I F Q F T K C C P P K P K D K S N L L
Y Q M E T V R S D S I I F Q F T K C C P P K P K D K S N L L
                    490                 500                 510
481  Y Q M E T V R S D S I I F Q F T K C C P P K P K D K S N L L
481  Y Q M E T V R S D N I I F Q F T K C C P P K P K D K S N L L

V F R T N G . S D V . . S P T L Q R . . . . N Q M V F H K I
V F R T N G V S D V Q I S P T L Q R H T N V N Q M V F H K I
                    520                 530                 540
511  V F R T N G I S D V Q I S P T L Q R H N N V N Q M V F H K I
511  V F R T N G V S D V P T S P T L Q R P T H M N Q M V F H K I

R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
                    550                 560                 570
541  R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y
541  R N E D L I F N E S L G Q G T F T K I F K G V R R E V G D Y

G Q L H . T E V L L K V L D K A H R N Y S E S F F E A A S M
G Q L H E T E V L L K V L D K A H R N Y S E S F F E A A S M
                    580                 590                 600
571  G Q L H K T E V L L K V L D K A H R N Y S E S F F E A A S M
571  G Q L H E T E V L L K V L D K A H R N Y S E S F F E A A S M
```

FIGURE 2C

```
      M S . L S H K H L V L N Y G V C V C G . E N I L V Q E F V K
      M S Q L S H K H L V L N Y G V C V C G D E N I L V Q E F V K
                    610                   620                   630

601   M S Q L S H K H L V L N Y G V C V C G E E N I L V Q E F V K
601   M S K L S H K H L V L N Y G V C V C G D E N I L V Q E F V K


      F G S L D T Y L K K N K N . I N I L W K L . V A K Q L A W A
      F G S L D T Y L K K N K N S I N I L W K L G V A K Q L A W A
                    640                   650                   660

631   F G S L D T Y L K K N K N S I N I L W K L G V A K Q L A W A
631   F G S L D T Y L K K N K N C I N T L W K L E V A K Q L A W A


      M H F L E E . . L I H G N V C A K N I L L I R E E D R . T G
      M H F L E E N S L I H G N V C A K N I L L I R E E D R K T G
                    670                   680                   690

661   M H F L E E K S L I H G N V C A K N I L L I R E E D R R T G
661   M H F L E E N T L I H G N V C A K N I L L I R E E D R K T G


      N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P
      N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P
                    700                   710                   720

691   N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P
691   N P P F I K L S D P G I S I T V L P K D I L Q E R I P W V P


      P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G
      P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G
                    730                   740                   750

721   P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G
721   P E C I E N P K N L N L A T D K W S F G T T L W E I C S G G


      D K P L S A L D S Q R K L Q F Y E D . H Q L P A P K W . E L
      D K P L S A L D S Q R K L Q F Y E D K H Q L P A P K W A E L
                    760                   770                   780

751   D K P L S A L D S Q R K L Q F Y E D K H Q L P A P K W T E L
751   D K P L S A L D S Q R K L Q F Y E D R H Q L P A P K W A E L


      A N L I N N C M D Y E P D F R P . F R A . I R D L N S L F T
      A N L I N N C M D Y E P D F R P A F R A V I R D L N S L F T
                    790                   800                   810

781   A N L I N N C M D Y E P D F R P A F R A V I R D L N S L F T
781   A N L I N N C M D Y E P D F R P S F R A I I R D L N S L F T
```

FIGURE 2D

```
        P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D
        P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D
                    820               830               840

811     P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D
811     P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D

        P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P
        P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P
                    850               860               870

841     P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P
841     P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P

        L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E
        L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E
                    880               890               900

871     L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E
871     L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E

        I L K S L Q H D N I V K Y K G V C Y S A G R R N L . L I M E
        I L K S L Q H D N I V K Y K G V C Y S A G R R N L K L I M E
                    910               920               930

901     I L K S L Q H D N I V K Y K G V C Y S A G R R N L R L I M E
901     I L K S L Q H D N I V K Y K G V C Y S A G R R N L K L I M E

        Y L P Y G S L R D Y L Q K H K E R I D H . K L L Q Y T S Q I
        Y L P Y G S L R D Y L Q K H K E R I D H I K L L Q Y T S Q I
                    940               950               960

931     Y L P Y G S L R D Y L Q K H K E R I D H K K L L Q Y T S Q I
931     Y L P Y G S L R D Y L Q K H K E R I D H I K L L Q Y T S Q I

        C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V
        C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V
                    970               980               990

961     C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V
961     C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V

        K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W
        K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W
                    1000              1010              1020

991     K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W
991     K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W
```

FIGURE 2E

```
      Y A P . S L T E S K F S V A S D V W S F G V V L Y E L F T Y
      Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y
                  1030                1040                1050
1021  Y A P Q S L T E S K F S V A S D V W S F G V V L Y E L F T Y
1021  Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y

      I E K S K S P P . E F M R M I G N D K Q G Q M I V F H L I E
      I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E
                  1060                1070                1080
1051  I E K S K S P P V E F M R M I G N D K Q G Q M I V F H L I E
1051  I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E

      L L K . N G R L P R P . G C P D E I Y . I M T E C W N N N V
      L L K S N G R L P R P D G C P D E I Y V I M T E C W N N N V
                  1090                1100                1110
1081  L L K S N G R L P R P E G C P D E I Y V I M T E C W N N N V
1081  L L K N N G R L P R P D G C P D E I Y M I M T E C W N N N V

      . Q R P S F R D L . . . . . . I . . . . .
      S Q R P S F R D L A L R V G Q I K D G T A G
                  1120                1130
1111  S Q R P S F R D L S F - - G W I K C G T V .
1111  N Q R P S F R D L A L R V D Q I R D N M A G
```

FIGURE 2F

DECLARATION AND POWER OF ATTORNEY FOR
UNITED STATES PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

A NOVEL HUMAN JAK2 KINASE

the specification of which

/ / is attached hereto.

/X/ was filed on <u>December 5, 1995</u> as application Serial No. <u>08/567,508</u> and if this box contains an / /, was amended on _____

/ / was filed as Patent Cooperation Treaty international application No. _____ on _____, 19__, if this box contains an X / /, was amended on under Patent Cooperation Treaty Article 19 on _____19_, and if this box contains an X / /, was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international applications(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

Country	Number	Filing Date	Priority Claimed
			/_/ Yes /_/ No
			/_/ Yes /_/ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and of any Patent Cooperation Treaty international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)

I hereby appoint the following:

BARBARA J. LUTHER	**Registration No.:**	**33,954**
DEBRA J. GLAISTER	**Registration No.:**	**33,888**

respectively and individually, as my attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

BARBARA J. LUTHER, ESQ.
INCYTE PHARMACEUTICALS, INC.
3174 PORTER DRIVE, PALO ALTO, CALIFORNIA 94304

TEL. : 415-855-0555   FAX: 415-852-0195

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

*IMPORTANT: *Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.*

**Sole Inventor or
First Joint Inventor:**

Full name: **ROGER COLEMAN**
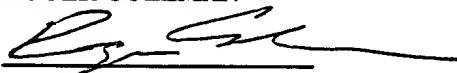
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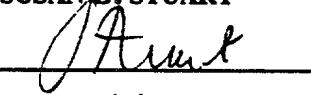
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: NOVEL HUMAN JAK2 KINASE

Serial No.: 09/196,480   Filing Date: November 19, 1998

Examiner: Hutson, R.   Group Art Unit: 1652

Assistant Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

CERTIFICATE UNDER 37 C.F.R. §3.73(b),
REVOCATION OF POWER OF ATTORNEY AND
APPOINTMENT OF NEW ATTORNEYS

Sir:

The undersigned has reviewed all the documents in the chain of title of the above-identified patent application and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

Incyte Pharmaceuticals, Inc., having a principal place of business located at 3174 Porter Drive, Palo Alto, California 94304, certifies that it is the assignee and owner of the entire right, title and interest in, to, and under the invention described and claimed in the above-identified application by virtue of an Assignment recorded at Reel 7984, Frame 0461, hereby revokes all previous powers of attorney and appoints the following patent attorneys/agents:

Narinder S. Banait	**Reg. No. 43,482**
Adam Warwick Bell	**Reg. No. 43,490**
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Lynn E. Murry	**Reg. No. 42,918**

48587   1   09/196,480

Danielle M. Pasqualone   Reg. No. 43,847
Susan K. Sather   Reg. No. 44,316
David G. Streeter   Reg. No. 43,168

Please direct all correspondence to:

<div align="center">

Legal Department
Incyte Pharmaceuticals, Inc.
3174 Porter Drive
Palo Alto, California 94304

</div>

and direct all telephone calls and facsimile transmissions to: ___Diana Hamlet-Cox___, Incyte Pharmaceuticals, Inc., Phone: (650) 855-0555, Fax: (650) 845-4166.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

<div align="center">

INCYTE PHARMACEUTICALS, INC.

</div>

Date: ___October 21, 1999___   By: _____
                                       Lee Bendekgey
                                       VP, General Counsel/Corporate Secretary

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Coleman, Roger
           Stuart, Susan G.

(ii) TITLE OF THE INVENTION: A NOVEL HUMAN JAK2 KINASE

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
  (A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
  (B) STREET: 3174 Porter Drive
  (C) CITY: Palo Alto
  (D) STATE: CA
  (E) COUNTRY: US
  (F) ZIP: 94304

(v) COMPUTER READABLE FORM:
  (A) MEDIUM TYPE: Diskette
  (B) COMPUTER: IBM Compatible
  (C) OPERATING SYSTEM: DOS
  (D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
  (A) APPLICATION NUMBER: 08/567,508
  (B) FILING DATE: 05-DEC-1995
  (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
  (A) APPLICATION NUMBER:
  (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
  (A) NAME: Billings, Lucy J.
  (B) REGISTRATION NUMBER: 36,749
  (C) REFERENCE/DOCKET NUMBER: PF-0049US

(ix) TELECOMMUNICATION INFORMATION:
  (A) TELEPHONE: 650-855-0555
  (B) TELEFAX: 650-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 4482 base pairs
  (B) TYPE: nucleic acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:. cDNA

(vii) IMMEDIATE SOURCE:
  (A) LIBRARY: Placenta
  (B) CLONE: 179527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCACGCGTCC GGTTGCCAAC CCGCAGGCGA CTGGGCGCTT CATCCCACCC TCACCCCTTT    60
CCAGCCAAGG TGGCTGATCG GAGTGCAGGCT CTCGAGGTCG CATTGCCACG AAACGGNGTG   120
TGTGAGCGCG TTGTCCCCGG NCCCCGGGGC CACTTCCCCT CGGCCTAGNA GACTGGACTG    180
GGGAAGGACG GGTCTGTTGT ACCCGGGAGG TGGAAGGAAA AGCCGAAAGC GGAGAAGTGT    240
GCGGGAGGGG AGTCTCCGCG CGGAGGNAGA CCGGNCTCCT CCAGTGCAGG TTGTGCGCTG    300
GGGAGCCAGC CASGGCAAAT GTTCTGAAAA AGACTCTGCA TGGGAATGGC CTGCCTTACG    360
ATGACAGAAA TGGAGGGAAC ATCCACCTCT TCTATATATC AGAATGGTGA TATTTCTGGA    420
AATGCCAATT CTATGAAGCA AATAGATCCA GTTCTTCAGG TGTATCTTTA CCATTCCCTT    480
GGGAAATCTG AGGCAGATTA TCTGACCTTT CCATCGGGG AGTATGTTGG AGAAGAAATC    540
TGTATTGCTG CTTCTAAAGC TTGTGGTATC ACACCTGTGT ATCATAATAT GTTTGCTTTA    600
ATGAGTGAAA CAGAAAGGAT CTGGTATCCA CCCAACCATG TCTTCCATAT AGATGAGTCA    660
ACCAGGCATA ATGTACTCTA CAGAATAAGA TTTTACTTTC CTCGTTGGTA TTGCAGTGGC    720
AGCAACAGAG CCTATCGGCA TGGAATATCT CGAGGTGCTG AAGCTCCTCT TCTTGATGAC    780
TTTGTCATGT CTTACCTCTT TGCTCAGTGG CGGCATGATT TTGTGCATGG ATGGATAAAA    840
GTACCTGTGA CTCATGAAAC ACAGGAAGAA TGTCTTGGGA TGACAGTGTT AGATATGATG    900
AGAATAGCCA AAGAAAACGA TCAAACCCCA CTGGCCATCT ATAACTCTAT CAGCTACAAG    960
ACATTCTTAC CACAATGTAT TCGAGCAAAG ATCCAAGACT ATCATATTTT GACAAGGAAG   1020
CGAATAAGGT ACAGATTTCG CAGATTTATT CAGCAATTCA GCCAATGCAA AGCCACTGCC   1080
AGAAACTTGA AACTTAAGTA TCTTATAAAT CTGGAAACTC TGCAGTCTGC CTTCTACACA   1140
GAGAAATTTG AAGTAAAAGA ACCTGGAAGT GGTCCTTCAG GTGAGGAGAT TTTTGCAACC   1200
ATTATAATAA CTGGAAACGG TGGAATTCAG TGGTCAAGAG GGAAACATAA AGAAAGTGAG   1260
ACACTGACAG AACAGGATTT ACAGTTATAT TGCGATTTTC CTAATATTAT TGATGTCAGT   1320
ATTAAGCAAG CAAACCAAGA GGGTTCAAAT GAAAGCCGAG TTGTAACTAT CCATAAGCAA   1380
GATGGTAAAA ATCTGGAAAT TGAACTTAGC TCATTAAGGG AAGCTTTGTC TTTCGTGTCA   1440
TTAATTGATG GATATTATAG ATTAACTGCA GATGCACATC ATTACCTCTG TAAAGAAGTA   1500
GCACCTCCAG CCGTGCTTGA AAATATACAA AGCAACTGTC ATGGCCCAAT TTCGATGGAT   1560
TTTGCCATTA GTAAACTGAA GAAAGCAGGT AATCAGACTG GACTGTATGC ACTTCGATGC   1620
AGTCCTAAGG ACTTTAATAA ATATTTTTTG ACTTTTGCTG TCGAGCGAGA AAATGTCATT   1680
GAATATAAAC ACTGTTTGAT TACAAAAAAT GAGAATGAAG AGTACAACCT CAGTGGGACA   1740
AAGAAGAACT TCAGCAGTCT TAAAGATCTT TTGAATTGTT ACCAGATGGA AACTGTTCGC   1800
TCAGACAATA TAATTTTCCA GTTTACTAAA TGCTGTCCCC CAAAGCCAAA AGATAAATCA   1860
AACCTTCTAG TCTTCAGAAC GAATGGTGTT TCTGATGTAC CAACCTCACC AACATTACAG   1920
AGGCCTACTC ATATGAACCA AATGGTGTTT CACAAAATCA GAAATGAAGA TTTGATATTT   1980
AATGAAAGCC TTGGCCAAGG CACTTTTACA AAGATTTTTA AAGGCGTACG AAGAGAAGTA   2040
GGAGACTACG GTCAACTGCA TGAAACAGAA GTTCTTTTAA AAGTTCTGGA TAAAGCACAC   2100
AGGAACTATT CAGAGTCTTT CTTTGAAGCA GCAAGTATGA TGAGCAAGCT TTCTCACAAG   2160
CATTTGGTTT TAAATTATGG AGTATGTGTC TGTGGAGACG AGAATATTCT GGTTCAGGAG   2220
TTTGTAAAAT TTGGATCACT AGATACATAT CTGAAAAAGA ATAAAAATTG TATAAATATA   2280
TTATGGAAAC TTGAAGTTGC TAAACAGTTG GCATGGGCCA TGCATTTTCT AGAAGAAAAC   2340
ACCCTTATTC ATGGGAATGT ATGTGCCAAA AATATTCTGC TTATCAGAGA AGAAGACAGG   2400
AAGACAGGAA ATCCTCCTTT CATCAAACTT AGTGATCCTG GCATTAGTAT TACAGTTTTG   2460
CCAAAGGACA TTCTTCAGGA GAGAATACCA TGGGTACCAC CTGAATGCAT TGAAAATCCT   2520
AAAAATTTAA ATTTGGCAAC AGACAAATGG AGTTTTGGTA CCACTTTGTG GGAAATCTGC   2580
AGTGGAGGAG ATAAACCTCT AAGTGCTCTG GATTCTCAAA GAAAGCTACA ATTTTATGAA   2640
GATAGGCATC AGCTTCCTGC ACCAAAGTGG GCAGAATTAG CAAACCTTAT AAATAATTGT   2700
ATGGATTATG AACCAGATTT CAGGCCTTCT TTCAGAGCCA TCATACGAGA TCTTAACAGT   2760
TTGTTTACTC CAGATTATGA ACTATTAACA GAAAATGACA TGTTACCAAA TATGAGGATA   2820
GGTGCCTTGG GGTTTTCTGG TGCCTTTGAA GACCGGGATC CTACACAGTT TGAAGAGAGA   2880
CATTTGAAAT TTCTACAGCA ACTTGGCAAG GGTAATTTTG GGAGTGTGGA GATGTGCCGG   2940
TATGACCCTC TACAGGACAA CACTGGGGAG GTGGTCGCTG TAAAAAAGCT TCAGCATAGT   3000
ACTGAAGAGC ACCTAAGAGA CTTTGAAAGG GAAATTGAAA TCCTGAAATC CCTACAGCAT   3060
GACAACATTG TAAAGTACAA GGGAGTGTGC TACAGTGCTG GTCGGCGTAA TCTAAAATTA   3120
ATTATGGAAT ATTTACCATA TGGAAGTTTA CGAGACTATC TTCAAAAACA TAAAGAACGG   3180
ATAGATCACA TAAAACTTCT GCAGTACACA TCTCAGATAT GCAAGGGTAT GGAGTATCTT   3240
GGTACAAAAA GGTATATCCA CAGGGATCTG GCAACGAGAA ATATATTGGT GGAGAACGAG   3300
AACAGAGTTA AAATTGGRGA TTTTGGGTTA ACCAAAGTCT TGCCACAAGA CAAAGAATAC   3360
TATAAAGTAA AAGAACCTGG TGAAAGTCCC ATATTCTGGT ATGCTCCAGA TCACTGACA   3420
GAGAGCAAGT TTTCTGTGGC CTCAGATGTT TGGAGCTTTG GAGTGGTTCT GTATGAACTT   3480
TTCACATACA TTGAGAAGAG TAAAAGTCCA CCAGCGGAAT TTATGCGTAT GATTGGCAAT   3540
GACAAACAAG GACAGATGAT CGTGTTCCAT TTGATAGAAC TTTTGAAGAA TAATGGAAGA   3600
TTACCAAGAC CAGATGGATG CCCAGATGAG ATCTATATGA TCATGACAGA ATGCTGGAAC   3660
AATAATGTAA ATCAACGCCC CTCCTTTAGG GATCTAGCTC TTCGAGTGGA TCAAATAAGG   3720
GATAACATGG CTGGATGAAA GAAATGACCT TCATTCTGAG ACCAAAGTAG ATTTACAGAA   3780
CAAAGTTTTA TATTTCACAT TGCTGTGGAC TATTATTACA TATATCATTA TTATATAAAT   3840
CATGATGCTA GCCAGCAAAG ATGTGAAAAT ATCTGCTCAA AACTTTCAAA GTTTAGTAAG   3900
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TTTTTCTTCA TGAGGCCACC AGTAAAAGAC ATTAATGAGA ATTCCTTAGC AAGGATTTTG    3960
TAAGAAGTTT CTTAAACATT GTCAGTTAAC ATCACTCTTG TCTGGCAAAA GAAAAAAAAT    4020
AGACTTTTTC AACTCAGCTT TTTGAGACCT GAAARAATTA TTATGTAAAT TTTGCAATGT    4080
TAAAGATGCA CAGAATATGT ATGTATAGTT TTTACCACAG TGGATGTATA ATACCTTGGC    4140
ATCTTGTGTG ATGTTTAACA CACATGAGGG CTGGTGTTCA TTAATACTGT TTTCTAATTT    4200
TTCCATGGTT AATCTATAAT TAATTACTTC ACTAAACAAA CAAATTAAGA TGTTCAGATA    4260
ATTGAATAAG TACCTTTGTG TCCTTGTTCA TTTATATCGC TGGCCAGCAT TATAAGCAGG    4320
TGTATACTTT TAGCTTGTAG TTCCATGTAC TGTAAATATT TTTCACATAA AGGGAACAAA    4380
TGTCTAGTTT TATTTGTATA GGAAATTTGC CCTGACCCTA AATAATACAT TTTGAAATGA    4440
AACAAGCTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAA AG                        4482
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(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1132 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (vii) IMMEDIATE SOURCE:
      (A) LIBRARY:
      (B) CLONE:

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr
 1               5                  10                  15
Ser Ser Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met
                20                  25                  30
Lys Gln Ile Asp Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly
            35                  40                  45
Lys Ser Glu Ala Asp Tyr Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly
        50                  55                  60
Glu Glu Ile Cys Ile Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65                  70                  75                  80
Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
                85                  90                  95
Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asn Val
            100                 105                 110
Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser Gly Ser
        115                 120                 125
Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu
        130                 135                 140
Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp
145                 150                 155                 160
Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
                165                 170                 175
Glu Cys Leu Gly Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu
            180                 185                 190
Asn Asp Gln Thr Pro Leu Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr
        195                 200                 205
Phe Leu Pro Gln Cys Ile Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
    210                 215                 220
Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225                 230                 235                 240
Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu Lys Tyr Leu Ile
                245                 250                 255
Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe Glu Val
            260                 265                 270
Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
        275                 280                 285
Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys
        290                 295                 300

Glu Ser Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe
305                 310                 315                 320
Pro Asn Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser
            325                 330                 335
Asn Glu Ser Arg Val Val Thr Ile His Lys Gln Asp Gly Lys Asn Leu
            340                 345                 350
Glu Ile Glu Leu Ser Ser Leu Arg Glu Ala Leu Ser Phe Val Ser Leu
        355                 360                 365
Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp Ala His His Tyr Leu Cys
    370                 375                 380
```

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Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile Gln Ser Asn Cys
385                 390                 395                 400
His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys Lys Ala
            405                 410                 415
Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
        420                 425                 430
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu
        435                 440                 445
Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu
    450                 455                 460
Ser Gly Thr Lys Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys
465                 470                 475                 480
Tyr Gln Met Glu Thr Val Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr
            485                 490                 495
Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
        500                 505                 510
Arg Thr Asn Gly Val Ser Asp Val Pro Thr Ser Pro Thr Leu Gln Arg
        515                 520                 525
Pro Thr His Met Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
    530                 535                 540
Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys Ile Phe
545                 550                 555                 560
Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr
            565                 570                 575
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu
        580                 585                 590
Ser Phe Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His
        595                 600                 605
Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu
    610                 615                 620
Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
625                 630                 635                 640
Asn Lys Asn Cys Ile Asn Ile Leu Trp Lys Leu Glu Val Ala Lys Gln
            645                 650                 655
Leu Ala Trp Ala Met His Phe Leu Glu Glu Asn Thr Leu Ile His Gly
            660                 665                 670
Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Lys
        675                 680                 685
Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
    690                 695                 700
Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
705                 710                 715                 720
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
            725                 730                 735
Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
        740                 745                 750
Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
        755                 760                 765
Arg His Gln Leu Pro Ala Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile
    770                 775                 780
Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ser Phe Arg Ala
785                 790                 795                 800
Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
            805                 810                 815
Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
        820                 825                 830
Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
        835                 840                 845
Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
    850                 855                 860
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
865                 870                 875                 880
Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
            885                 890                 895
Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
            900                 905                 910
Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile
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            915                        920                    925
Met Glu Tyr Leu Pro Tyr Gly Ser Leu Arg Asp Tyr Leu Gln Lys His
    930                        935                    940
Lys Glu Arg Ile Asp His Ile Lys Leu Leu Gln Tyr Thr Ser Gln Ile
945                    950                        955            960
Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
                965                    970                    975
Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
            980                    985                    990
Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr
        995                    1000                   1005
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Glu
    1010                   1015                   1020
Ser Leu Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe
025                    1030                   1035                1040
Gly Val Val Leu Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser
                1045                   1050                   1055
Pro Pro Ala Glu Phe Met Arg Met Ile Gly Asn Asp Lys Gln Gly Gln
            1060                   1065                   1070
Met Ile Val Phe His Leu Ile Glu Leu Leu Lys Asn Asn Gly Arg Leu
        1075                   1080                   1085
Pro Arg Pro Asp Gly Cys Pro Asp Glu Ile Tyr Met Ile Met Thr Glu
    1090                   1095                   1100
Cys Trp Asn Asn Asn Val Asn Gln Arg Pro Ser Phe Arg Asp Leu Ala
105                    1110                   1115                1120
Leu Arg Val Asp Gln Ile Arg Asp Asn Met Ala Gly
            1125                   1130
```

(2) INFORMATION FOR SEQ ID NO:3:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 1129 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Ala Thr Ser Thr
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Ser Pro Val His Gln Asn Gly Asp Ile Pro Gly Ser Ala Asn Ser Val
            20                  25                  30
Lys Gln Ile Glu Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly
        35                  40                  45
Gln Ala Glu Gly Glu Tyr Leu Lys Phe Pro Ser Gly Glu Tyr Val Ala
    50                  55                  60
Glu Glu Ile Cys Val Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65              70                  75                  80
Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
                85                  90                  95
Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asp Ile
            100                 105                 110
Leu Tyr Arg Ile Arg Phe Tyr Phe Pro His Trp Tyr Cys Ser Gly Ser
        115                 120                 125
Ser Arg Thr Tyr Arg Tyr Gly Val Ser Arg Gly Ala Glu Ala Pro Leu
    130                 135                 140
Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Val Gln Trp Arg His Asp
145             150                 155                 160
Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
                165                 170                 175
Glu Cys Leu Gly Met Ala Val Leu Asp Met Met Arg Ile Ala Lys Glu
            180                 185                 190
Lys Asp Gln Thr Pro Leu Ala Val Tyr Asn Ser Val Ser Tyr Lys Thr
        195                 200                 205
Phe Leu Pro Lys Cys Val Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
    210                 215                 220
Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225             230                 235                 240
Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu Lys Tyr Leu Ile
            245                 250                 255
Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Gln Phe Glu Val
            260                 265                 270
Lys Glu Ser Ala Arg Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
        275                 280                 285
Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys
        290                 295                 300
Glu Ser Glu Thr Leu Thr Glu Gln Asp Val Gln Leu Tyr Cys Asp Phe
305                 310                 315                 320
Pro Asp Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Cys Ser
                325                 330                 335
Asn Glu Ser Arg Ile Val Thr Val His Lys Gln Asp Gly Lys Val Leu
            340                 345                 350
Glu Ile Glu Leu Ser Ser Leu Lys Glu Ala Leu Ser Phe Val Ser Leu
            355                 360                 365
Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp Ala His His Tyr Leu Cys
    370                 375                 380
Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile His Ser Asn Cys
385                 390                 395                 400
His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys Lys Ala
                405                 410                 415
Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
            420                 425                 430
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu
```

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                   435                           440                           445
      Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Gly Glu Tyr Asn Leu
          450                           455                           460
      Ser Gly Thr Asn Arg Asn Phe Ser Asn Leu Lys Asp Leu Leu Asn Cys
      465                           470                           475                           480
      Tyr Gln Met Glu Thr Val Arg Ser Asp Ser Ile Ile Phe Gln Phe Thr
                               485                           490                           495
      Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
                           500                           505                           510
      Arg Thr Asn Gly Ile Ser Asp Val Gln Ile Ser Pro Thr Leu Gln Arg
                   515                           520                           525
      His Asn Asn Val Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
              530                           535                           540
      Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys Ile Phe
      545                           550                           555                           560
      Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Lys Thr
                           565                           570                           575
      Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu
                       580                           585                           590
      Ser Phe Phe Glu Ala Ala Ser Met Met Ser Gln Leu Ser His Lys His
                   595                           600                           605
      Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Glu Glu Asn Ile Leu
              610                           615                           620
      Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
      625                           630                           635                           640
      Asn Lys Asn Ser Ile Asn Ile Leu Trp Lys Leu Gly Val Ala Lys Gln
                           645                           650                           655
      Leu Ala Trp Ala Met His Phe Leu Glu Glu Lys Ser Leu Ile His Gly
                       660                           665                           670
      Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Arg
           675                           680                           685
      Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
              690                           695                           700
      Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
      705                           710                           715                           720
      Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
                           725                           730                           735
      Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
                       740                           745                           750
      Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
                   755                           760                           765
      Lys His Gln Leu Pro Ala Pro Lys Trp Thr Glu Leu Ala Asn Leu Ile
              770                           775                           780
      Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ala Phe Arg Ala
      785                           790                           795                           800
      Val Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
                           805                           810                           815
      Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
                       820                           825                           830
      Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
                   835                           840                           845
      Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
              850                           855                           860
      Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
      865                           870                           875                           880
      Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
                           885                           890                           895
      Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
                       900                           905                           910
      Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Arg Leu Ile
                   915                           920                           925
      Met Glu Tyr Leu Pro Tyr Gly Ser Leu Arg Asp Tyr Leu Gln Lys His
              930                           935                           940
      Lys Glu Arg Ile Asp His Lys Lys Leu Leu Gln Tyr Thr Ser Gln Ile
      945                           950                           955                           960
      Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
                           965                           970                           975
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Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
          980                     985                 990
Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr
          995             1000                1005
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Gln
    1010                 1015                1020
Ser Leu Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe
025                 1030                1035                1040
Gly Val Val Leu Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser
              1045                1050                1055
Pro Pro Val Glu Phe Met Arg Met Ile Gly Asn Asp Lys Gln Gly Gln
          1060                1065                1070
Met Ile Val Phe His Leu Ile Glu Leu Leu Lys Ser Asn Gly Arg Leu
          1075                1080                1085
Pro Arg Pro Glu Gly Cys Pro Asp Glu Ile Tyr Val Ile Met Thr Glu
    1090                 1095                1100
Cys Trp Asn Asn Asn Val Ser Gln Arg Pro Ser Phe Arg Asp Leu Ser
105                 1110                1115                1120
Phe Gly Trp Ile Lys Cys Gly Thr Val
              1125
```

          (2) INFORMATION FOR SEQ ID NO:4:

              (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 24 base pairs
                  (B) TYPE: nucleic acid
                  (C) STRANDEDNESS: single
                  (D) TOPOLOGY: linear

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGCGGAAGT GCTCTCGGCG GAAG   24

          (2) INFORMATION FOR SEQ ID NO:5:

              (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 24 base pairs
                  (B) TYPE: nucleic acid
                  (C) STRANDEDNESS: single
                  (D) TOPOLOGY: linear

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTGTGCTAC AGTGCTGGTC GTCG   24